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Cover picture

An enlarged silhouette of a copepod, *Haloptilus* sp. (2.5 mm long). The use of high-speed silhouette photography to characterise zooplankton is described on page 50.

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Advanced techniques in routine use at The Radiochemical Centre

Described below are just two examples of the many up-to-date techniques, which have been pioneered or applied for routine use at The Radiochemical Centre. These developments are part of our constant endeavour to maintain our position at the forefront of the specialised field of tracer methodology, so that we can continue our supply of radiochemicals of the highest quality and technical specifications.

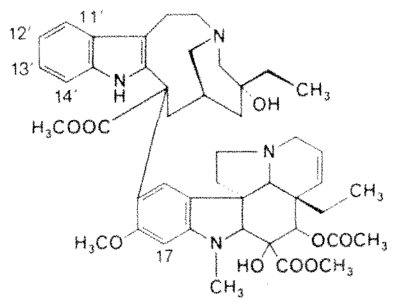
Distribution of labelling in tritium compounds

Modern techniques for the production of tritiated compounds are more sophisticated than those used in the early days of tritium labelling, and produce compounds labelled in specific positions rather than generally labelled. Nevertheless, it is necessary for many tracer applications of tritium compounds to know the *precise* position and configuration of the tritium labels. Traditional chemical methods of doing this are tedious and time consuming and subject to considerable error, and so the routine supply of such information has until recently not been possible.

The Radiochemical Centre, in collaboration with the University of Surrey, has developed over the past eight years the technique of tritium nuclear magnetic resonance (tnmr) spectroscopy for this purpose. This method is much quicker and more accurate than the traditional chemical or biochemical methods for determining distribution of tritium labelling.

It is now used routinely to establish the distribution of tritium labelling produced by the usual methods of tritiation employed at The Radiochemical Centre. We supply accurate details as to the position and configuration of the tritium labels for an increasing number of our labelled compounds.

A number of examples are given below:



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12′,13′ ..... 46% 14′ 2%

[1β, 2β(n)-^{3}H]TESTOSTERONE
Code TRK.162
1α ..... 5.9% 2α 8.2%
1β ..... 46.0% 2β 39.9%

[6-^{3}H]BENZO[a]PYRENE
Code TRK.501
6 ..... >95% 1 ..... <5%

D-[2-^{3}H]GLUCOSE
Code TRK.361
2(α) ..... 40% 2(β) 60%

L-[3,4(n)-^{3}H]VALINE
Code TRA/K.533
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3 .... 28%

The latest of our publications is as follows:
AL-RAWI, J.M.A., BLOXSIDE, J.P., ELVIDGE, J.A., JONES, J.R., CHAMBERS, V.E.M., CHAMBERS, V.M.A. and EVANS, E.A., *Steroids* vol. 28 (3), p.p. 359-375, 1976.

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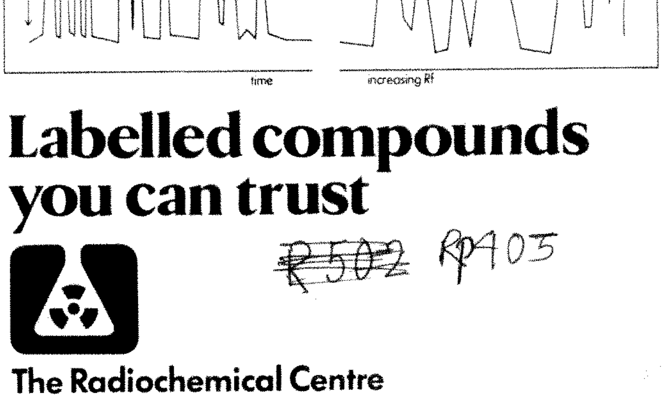
The example illustrated below illustrates the clear superiority of HPLC when used as an analytical tool. The mixture used comprised the tritium labelled mono-, di- and triphosphates of adenosine, cytidine, guanosine and uridine, and all are clearly separated in the HPLC system.



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- Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.
- 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

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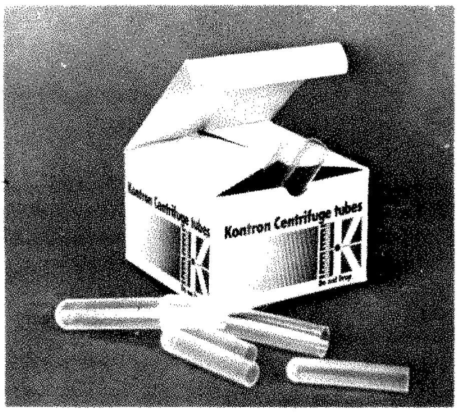

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UNCSTD's Year

1979 is the year of UNCSTD, the United Nations Conference on Science and Technology for Development, which will meet in Vienna for two weeks during August. In this past year almost every nation with any interest in science and technology has compiled a national paper representing its point of view, and well over 100 documents, many running to 50 pages or more, now reside in the UNCSTD secretariat at the UN headquarters in New York. During these next few months the secretariat will have the unenviable task of distilling an immense amount of fact and opinion into some sort of agenda for Vienna. In the meantime subconferences in preparation for the main meeting continue unabated; in January for instance there will be meetings in Tallin in the Soviet Union and in Singapore (organised by the International Council of Scientific Unions).

Is the conference going to work out satisfactorily? What, even, do we mean by a 'satisfactory' conference? Presumably one that breaks up in fist fights or that falls apart over issues peripheral to the main themes would be deemed unsatisfactory. So too, although this view is not universally shared, would be one in which no voices were raised, no feeling engendered, no criticism voiced. Between these two poles there is much scope and it will depend largely on the leadership that the UNCSTD secretariat gives whether much of value emerges. The outcome of the labours of thousands who have contributed in one way or another to conference documents is, in the strange system in which we work, ultimately dependent on a handful of people at headquarters. In the past year such news that has filtered out has suggested quite strongly that all is not well. If relations are not all they ought to be, then this may well be excessively occupying the time of those who would be better employed in making the conference work.

But what of the papers themselves? After all the very act of compiling national papers must have put some countries through a salutary exercise of asking questions, perhaps for the first time, about the relationship between science, technology and development. We expressed the hope a year ago that this exercise would not be confined to bureaucrats but would encompass as many scientists and technologists as possible, whilst recognising that the conference is not specifically about science and technology as such. It is pleasing to be able to note that if the preambles to papers are to be believed a widespread process of consultation, meetings and conferences has gone on in many developing countries. Developed countries, cautious about committing themselves to large new expenditures on major institutional change, have on the whole provided papers descriptive of what they can do and are prepared to do but which shy away from more general discussions of principles—some also show signs of having little involved people outside certain official circles. The British paper is a case in point; it is scrupulously accurate but the Overseas Development Ministry, which compiled it has not in any sense used the preparation of the document as an opportunity to get a national debate going.

The Vienna meeting could be an excellent opportunity for the world to evaluate very carefully just what is being and could be done in the field of science, technology and development. But for this to happen some very self-critical voices would need to be raised. Unfortunately those who attend are most likely to be representatives of government agencies and national science funding agencies with a natural concern for self-preservation. Even if the papers contain an element of self-criticism (and a few do, notably from the developing world), when it comes to the meeting itself it will take a very brave delegate to acknowledge publicly—and take the message back home—that national mechanisms for channelling science and technology into development are in need of overhaul. And yet this undoubtedly needs saying. In the developed world there is inadequate discussion about whether the level of scientific resources devoted to the developing world is appropriate and whether present organisations are making the best use of these resources. In the developing world national science bodies too often seem to get into the hands of scientists who steer the country's effort away from the immense complexities of development and towards the slightly less complex business of imitating Western science.

If the UNCSTD secretariat could make delegates ask fundamental questions about their own national efforts, that would be a real achievement. And if the organisers of the peripheral meetings in Vienna could reach out beyond official viewpoints and tap some of the more speculative feeling abroad, then the whole exercise could still be very worthwhile. □

Kenya leads the way in environmental planning

THE government of Kenya has started a systematic across-the-board environmental assessment of its entire development programme—the first national exercise of its kind in the Third World. Officials at the United Nations Environment Programme (UNEP) and the United Nations Development Programme (UNDP), both of which are assisting Kenya in executing the project, are enthusiastic about this project.

Its success should help UNDP to promote similar environmental assessment projects in other developing countries, where many policy makers continue to harbour the same mistrust towards the environmental debate that they displayed at the Stockholm conference in 1972. While many argue that environmental considerations are a luxury for Third World development planners, there are others who see in environmentalism a philosophy designed to retard the economic development of the Third World, particularly its industrial development.

The Kenyans are quite aware that there can be strong conflicts between environment and development. They are therefore looking for only limited but quite realistic results from their exercise. At the least, it should help to reduce those innumerable environmental problems that are often created because of simple oversights at the planning stage.

Kenya's Tana River development scheme, for instance, will create one of the biggest man-made lakes in Africa. The spread of irrigation carries the threat of schistosomiasis. Between disease and agricultural prosperity Kenya, like any other Third World country, would obviously choose the latter. However, an advance environmental assessment exercise would help to promote possible control measures right from the beginning. It would also remind project authorities that the labour force building the dam has to be provided with energy. Otherwise, merely because of this oversight, forests could get depleted, and the resulting soil erosion could lead to rapid silting of the new reservoir.

Environmentally bad siting of projects, like a chemical plant near a bird sanctuary, can be prevented quite easily with the help of such exercises. "To delay the starting of a paper mill after it has been built, neither helps the country nor the firm setting it up", explains George Muhoho, until recently head of Kenya's National Environment Secretariat (NES), the Kenyan agency co-ordinating the environment-development project.

In the past, the NES has conducted several studies on various individual projects and influenced government decisions. But the current exercise now provides it with a rare opportunity to develop a comprehensive and long-term approach to environmental planning in the country.

The final recommendations of the project may well strongly favour a high rate of industrial growth. The most pressing environmental issues in Kenya are those related to land-use. South of the Sahara, Kenya has the highest population growth rate. Kenya's population is expected to double within 20 years. At the moment, nearly 90% of Kenya's population lives in the rural areas earning its livelihood predominantly from agriculture. Unless a rapid transformation takes place in the occupational structure of the country and more people find jobs in industry, the pressure on the land could become too intense.

As increasingly marginal land is brought under cultivation, deforestation and desertification will increase. Encroachment on game parks could seriously affect wildlife conservation. By contrast, Kenya could simply forget about air pollution control until it has reached a far higher level of industrial development. A comprehensive environmental planning exercise could thus identify urgent development priorities and critical environmental issues as well.

An important aspect of the project is that it relies largely on local Kenyan expertise. The temptation to invite a UN mission consisting of Western experts to prepare a report on environmental problems was studiously avoided. Such an approach would have robbed the Kenyans of a chance to assess by themselves the environmental effects of what they were doing, and thus develop their own perspective on environment and development related issues. Moreover, even in the West, few people can really stake a claim to be an expert on environment-development planning.

To ensure the involvement of all available local expertise, and popular participation, the project is being executed in three phases. In the first phase, which was completed in August, 1978, five papers were prepared that surveyed major environmental problems in Kenya. These papers were then thrown open for discussions at a week-long meeting in July, 1978, attended by 11 senior Kenyan officials, including the Minister of Finance and Planning, and several permanent secretaries in the government. This meeting identified existing gaps in knowledge and possible policy alternatives. Finally, a steering committee for the rest of the project was formed consisting of the officials present at the meeting to enlist the active co-operation of all the ministries.

In the second phase, much more specialised studies on specific scientific, technical, legal and sociological issues are being conducted to find answers to the questions raised at the earlier meeting. To do these studies, local expertise will be drawn from wherever available—from official institutions to non-government organisations and universities. Foreign experts will be recruited only for highly technical subjects where no local expertise exists. Finally, in the third phase, to be completed by August, 1979, the final recommendations will be put together.

Anil Agrawal

The pressure on land: environmental oversights can limit growth

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Bacteriologists lobby GMAG's first public meeting

Sir William Henderson: optimistic

A LOBBY for the exemption of experiments in which recombinant DNA techniques are used simply to rearrange the genes within *E. coli* and other bacteria made a strong protest against continuing control at GMAG's first public meeting last week.

GMAG, Britain's Genetic Manipulation Advisory Group, was fulfilling its promise to hold open meetings on its proposed new system for the risk assessment of recombinant DNA experiments (*Nature* **276,** 104; 1978).

The new chairman of GMAG, Sir William Henderson (above right), opened the meeting optimistically, saying that those who feel that GMAG's hand is too heavy "do not sufficiently allow for the depth of public interest, and how the composition of GMAG takes this into account". But all on GMAG, said Sir William "wish to improve the procedures and consult".

Those at the meeting, held on the last working day before Christmas (22 December), were mostly scientists. The consultation amounted largely to two protests from the floor: that the new scheme was difficult to operate in practice because of lack of sufficient knowledge; and that the re-arrangement of genes within a bacterium such as *E. coli* using recombinant DNA techniques (but not otherwise) must still legally be notified to the Health and safety Executive and then to GMAG, and must be carried out under Category I conditions.

These conditions, although the least stringent of GMAG's four categories of physical containment, are still more rigorous than normal laboratory practice and can mean that the laboratory has to be modified. This type of experiment has now been exempted from control under the proposed new NIH guidelines in the US, but hopes that the new proposals in the UK would automatically exempt these experiments have been disappointed.

Question after question from the floor drew an acknowledgment from Professor Mark Richmond of GMAG that the group would have to make a decision on this problem soon. John Maddox, one of GMAG's public interest representatives, defended the categorisation and notification of such experiments on political grounds. While accepting that the procedure for notification would be made simpler and easier, he took the view that public concern would be lessened if GMAG were seen to be controlling all experiments using recombinant DNA techniques, even though there might be scientific grounds for exemptions in certain cases.

From the floor Professor R. Pritchard warned that this type of apparent illogicality undermined the credibility of the whole notification procedure in the eyes of researchers and their local safety committees.

Nevertheless, the feeling of the meeting was that the new risk assessment scheme was more rational than previous guidelines. But its precise application worried people.

The most immediate problem is posed by the 'gaps' in biological knowledge which become apparent when a consistent attempt is made to calculate the risk of an experiment. For example, the causes of pathogenicity are not well understood, so one cannot estimate all the possible biological effects of a modified bacterium on the human body.

In cases like this Dr Sydney Brenner —who invented the scheme and was present at the meeting—falls back on averages. Of all known bacteria, what fraction are pathogenic? This fraction could be applied as the probability that any new bacterium were pathogenic.

Dr Peter Rigby of Imperial College, who according to Dr Brenner "has worked out a magnificent set of examples", raised the final issue: that all the calculation is in vain if the precise containment effectiveness of the four containment categories is unknown. Some claim the containment factor between categories I and IV to be 10^{12}; some 10^{8}. "It must be measured" said Dr Rigby, extracting a cautious commitment that GMAG act.

Eleanor Lawrence

UK health ann safety: £1,800 million bill

PREVENTION rather than cure, and hence research, is a major theme in the recently published annual reports of the UK's Health and Safety Commission (HSC) and Health and Safety Executive (HSE). The HSC's plans for the next five years, its report says, include epidemiological studies on populations at risk from hazardous substances; a major review of the risks to health from exposure to asbestos; the development of a policy for protecting people from the hazards of genetic engineering and working with dangerous pathogens; and research on the design safety of equipment in nuclear power reactors.

In the introduction to the HSE report John Locke, the HSE's Director General says that "research, the systematic collection and analysis of a wide range of data and the dissemination of information to all who need it" is the hallmark of the executive's efforts to reduce occupational hazards. He points out that the 16 million days lost in 1976 through accidents are estimated to have cost the country some £1,800 million. The "great majority of accidents could be prevented if simple precautions are taken" he says.

Over the past few years, the HSC's report says, there has been a reduction in the number of deaths from industrial accidents. Nevertheless, Mr Bill Simpson, Chairman of the commission, believes that "there is no room for complacency". Although the number of deaths has gone down, the number of notifiable accidents was 329,000 in 1977, 2,000 up on the previous year.

The commission's report also describes the preparation of draft regulations on the notification, and in some cases survey, of installations constituting a major hazard. It comments on the regulations laid before Parliament for the packaging and labelling of about 800 dangerous chemicals in common use and discusses its efforts to change the law which exempts government controlled institutions from prosecution under health and safety legislation.

The problem of the universities and health and safety is still under review by the executive according to John Locke. The HSE is still getting to know people in the universities (heads of departments, trade unionists etc.) and identifying those sections which may present special hazards to workers or the public. Locke feels that students are the main problem because generally they are not sufficiently well trained to work in hazardous environments.

Alastair Hay

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Protests over Einstein statue

A PROPOSAL by the US National Academy of Sciences to erect a 21-foot statue of Albert Einstein in its Washington grounds has run into a storm of protest from members of both the academy itself and of the local Washington community.

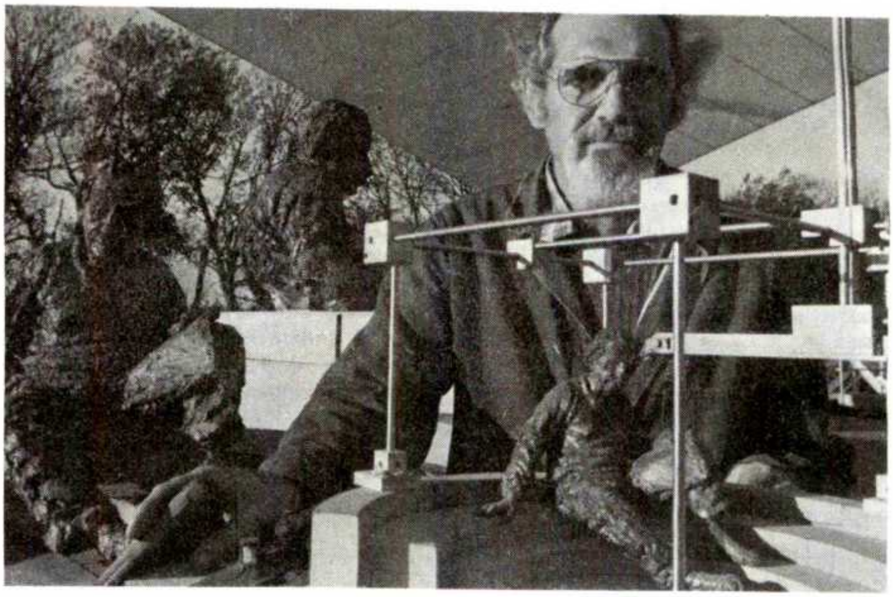
Several physicists have complained that at a time when basic science is being squeezed for funds, it is inappropriate for the academy to be seen spending $1.5 million on the statue, and that Einstein himself would have preferred the money to have been spent on some scientific endeavour.

Others have pointed out that Einstein repeatedly asked that he should not be treated as a cultural hero. And comments on the design of the statue in the local press have ranged from "a blunder of mediocrity" to "the latest disgrace to our city".

The decision to erect the statue as part of this year's centennial celebrations of Einstein's birth was announced two months ago by Dr Philip Handler, president of the academy. According to Dr Handler, the large size of the bronze statue—which together with its marble base will weigh 139 tons—represents that fact that Einstein was "a giant among men".

The sculpture is the work of artist Robert Berks, who sculpted a head of the physicist in 1953. He had previously proposed unsuccessfully a full-length statue to the Weizmann Institute in Israel, to Princeton University, and to the Smithsonian Institution in Washington.

The academy has written to all its members, as well as other members of the scientific and technical community, requesting financial support for the project. But while some have been pleased to contribute—over 2,000 contributions have so far been received—others are less happy.



Sculptor Robert Berks with models of the controversial statue

"There are a number of people here who almost hit the ceiling when they were asked to contribute to the statue. So far I have not heard a favourable word from anyone on the scheme," a physicist at one West coast university said last week.

Another complained that the decision to erect the statue had "simply arrived out of the blue", and that although the proposal had initially been put to and accepted by an annual meeting of the academy, there should have been wider discussion among members.

In Washington, opposition to the statue has been expressed mainly in the pages of the *Washington Post*. Following a critical article by one of the newspaper's contributors, a number of readers wrote in voicing objections.

Commenting on the large scale of the statue, for example, one correspondent wrote that "If anything concise may be said about Einstein's life, it is that he was a philosopher of mathematical precision and theoretical proportion". The *Post* itself joined in the fray with an editorial complaining that the statue "doesn't honour its subject", and suggesting that the academy should "start again".

A spokesman for the NAS said last week that the academy had not yet decided whether to reply to the criticism made in the *Post*, and pointed out that a large number of favourable comments about the statue—including its initial approval by Washington's Commission of Fine Arts—had been received.

David Dickson

AAAS to assist China with the popularisation of science

AN agreement opening the way to joint activities on the popularisation of science and on science education has been reached between the American Association for the Advancement of Science and the Scientific and Technical Association (STA) of the People's Republic of China.

The agreement, which is expected to lead to increasing co-operation at the nongovernmental level between the two organisations, was worked out during a visit to China last month by a 12-person delegation from the AAAS.

In particular, the agreement provides for a return visit by the Chinese to the US next spring, and for the AAAS to identify US specialists willing to visit China to lecture on topics specified by the STA.

Announcing these moves in Washington on the AAAS delegation's return, Mr Emilio Q. Daddario, chairman of the association's board of directors, said that various Chinese ministers had given their approval to such an agreement, and that President Carter's announcement of the normalisation of diplomatic relations between the two countries "cannot but help to make our agreement more meaningful."

Mr Daddario also said that the Chinese were contemplating sending a special misssion to the US to look at various ways in which science is "popularised". Such a visit might include talks with science journalists, and visits to science museums and technical information centres.

"During the Cultural Revolution there was a sense in China that science produces an intellectual elite. Now they want to make it clear to the masses that science has benefits for people, and that it is important that they should understand it", said Mr William Carey, executive officer of the AAAS.

"The Chinese want to get people in the streets, in the farms and in the factories to support the thrust towards modernisation in the fields of science and technology. And they have a very great appetite for US experts to go and give lectures, a method they are seizing upon to get a quick turn-around on their scientific efforts."

Mr Carey said that the Chinese had expressed interest in working with the AAAS on various aspects of science education, in particular the development of curricula for university and higher education.

David Dickson

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University of California faces drastic cuts

Proposed budget reductions may force closure of whole campuses and medical schools, writes **John H. Douglas** from San Francisco

RESPONDING to strong popular sentiment against high levels of government spending, California governor Edmund G. Brown, Jr., has demanded that all state agencies submit a list of programmes that can be cut immediately in order to bring about a 10% reduction in next year's budget.

Such cuts could drastically affect the prestige and research capability of the 9-campus University of California, which accepts students from the top 12.5% of the state's high school graduates. More students, however, would be affected by reductions in the 19-campus State University and Colleges system, which accepts students from the top one-third of high school classes, and in the 106 state-supported Community Colleges, which presently are open to any resident 18 years of age or older.

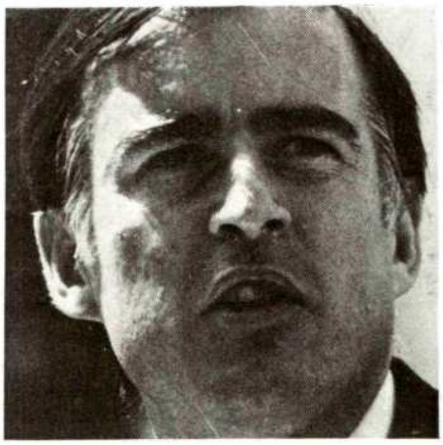
In a strongly worded reply to the governor's demands, University of California president David S. Saxon said flatly that spending reductions of this magnitude simply could not be accomplished by next year if the university were to honour its commitments to present students and to contracts with external agencies. Even to reduce the budget over the next three to five years, he said, would require closing a combination of campuses, medical schools and associated research institutes.

However, one of the factors cited by Governor Brown in defence of his proposed cuts is that during his past four year term the budget for the State University and Colleges has risen by 44.4% over the cost of living, although enrolment has dropped 2.6%. Such declines can be expected to continue, since a 25% reduction in the college-age population is expected over the next decade.

Saxon nevertheless warned the governor that to cut 10% (or about $77 million) in state support would translate into a much larger reduction in the overall university budget, since many programmes receive an equal amount of funding from other sources. Especially hard hit would be the science programmes that receive large research grants from the Federal government. "The number of jobs lost would be more than doubled", Saxon said.

To make matters even worse, the proposed reductions would come just at a time when the university has already suffered a $31 million cut in its proposed budget, following passage of Proposition 13 in this year's elections (which substantially reduced property taxes). And university employees did not receive any salary increases this year, putting them at the mercy of 8% inflation and setting the university a year behind many other institutions. Unless salary increases come soon, says Saxon, "our base would be so low that we would be unable to offer competitive salaries to any employees, and especially faculty, for the foreseeable future".

If major units of the University of California must be closed, speculation has centred on the vulnerability of the two smallest non-specialised campuses, at Riverside and Santa Cruz. The campuses suffered enrolment decreases this year of 5% and 3.5% respectively, compared to an increase in overall university enrolment of 1.5%. Riverside was upgraded from an agricultural institute and has never enjoyed the prestige of older campuses. Santa Cruz is remotely located and uses a controversial student evaluation system. Closing either unit would require special legislation, however.



Governor Brown : cuts all round

Other solutions under discussion would also require new legislation and, perhaps more important, the cooperation of the governor. Rather than cutting expenses, as directed, the university could be made more independent of state treasury funds by increasing student fees. California now supports the only system of higher education in the United States that is theoretically tuition free, although students pay about $700 a year in "fees". Increasing such fees by $100 a quarter (or imposing tuition of that amount) could raise nearly $35 million for the university. Proponents of this idea point out that more long-term loans are now available to middle class families than when the tuition-free system was originally set up. Raising admissions standards might also help relieve pressure on the university.

Population decreases and rising fees are likely to hit hardest at the Community Colleges, where competition for entrance is not so keen. Enrolment in normal credit courses declined 4% this year, although it was originally expected to rise 3%. And when new fees were added last fall to non-credit courses (which attract older students not working toward a degree), enrolment in such courses plunged 40%.

All of these problems are likely to come to a head next spring when the California legislature considers the state budget. The eventual outcome is likely to hinge on details of a political process that may seem quaint even by California's arcane standards. Although the universities and colleges have powerful friends in the legislature, the governor has authority to veto *specific* budget items and could thus eliminate individual programmes he dislikes. And, for the first time in this century, California's governor and lieutenant governor belong to different parties. The new Republican lieutenant governor, Mike Curb, has strongly hinted he will oppose Democrat Brown on the university issue. What this would mean remains unclear.

Finally, several legal questions remain unresolved. By custom, faculty tenure at the University of California has been considered to apply only on one campus. If campuses were eliminated, the future of tenured faculty would be cast into doubt, as would the university's reputation as a desirable institution with which to affiliate. Long-term research contracts between university institutes and Federal funding agencies would also be threatened.

In assessing the possible impact of the proposed budget cuts, some of the most vigorous and articulate support for the University of California has come from the president of a competing private institution: Richard W. Lyman of Stanford University. Calling the University of California system "the greatest publically supported university in the country", Lyman said: "Californians have gone to considerable lengths to try to protect this uniquely fine university from being converted into a mere arm of government. It would be a sad irony indeed if, in the effort to trim government down to defensible size, the university were to be damaged in ways that would require many years of painstaking work to correct, if indeed they could be remedied at all". □

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news in brief

NASA abandons attempts to rescue Skylab: The US National Aeronautics and Space Administration announced last week that it was abandoning its efforts to prevent Skylab, the 85-ton space station last used by astronauts in 1974, from falling to earth sometime next autumn.

As a result, the vehicle is expected to break up on entering the earth's atmosphere, scattering between 400 and 500 pieces of metal over a path 4,000 miles long. Some of these will probably strike the earth's surface, but according to the agency "the probability of injury or damage is less than that from meteorites."

Initially planned to remain in orbit until 1983, when it could be reached from a standard space shuttle flight, concern about Skylab's fate arose earlier this year when it was realised that the vehicle was slowing down as a result of the extra drag caused by sun-spot activity, and thus beginning to fall back to earth earlier than anticipated.

NASA had hoped that it could maintain Skylab in orbit until a small booster rocket could be sent up on an early space shuttle mission to take it into a higher orbit. However, problems with Skylab's control equipment, as well as development problems with the space shuttle programme, led to NASA's decision—supported by President Carter—to call off the rescue attempt.

Carter expected to boost defence research and cut back biomedical research: A major increase in financial support for military research and development, and a reduction in funding for biomedical research, are expected to be included in the budget request for the financial year 1980 which President Carter will present to Congress on 22 January.

The research and development budget is therefore expected to reflect the main thrusts of the overall budget, with reductions in social welfare programmes in an attempt to keep the budget deficit below $30 billion, but an increase in defence spending.

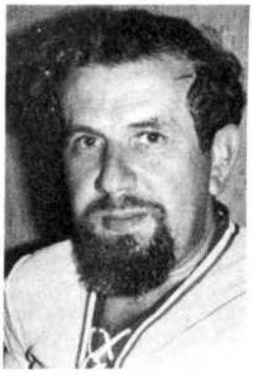
It is thought that total funding for military R & D will increase from $12.6 billion in the current financial year to over $13.5 billion. Within this figure, about $3 billion will be allocated to basic research, an increase of 15% over this year, and 7% above the inflation rate.

One administration official is quoted in *Business Week* as saying that "one of the very few certainties about the new budget right now is that basic research and exploratory development money will be downright abundant."

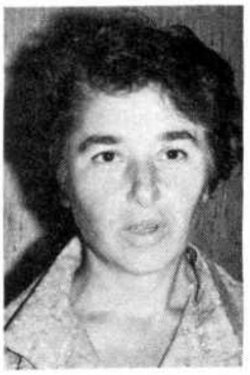
As far as biomedical research is concerned, the Office of Management and Budget is reported to have recommended that the budget of the National Institutes of Health be reduced from its current level of $3.25 billion to $3.03 billion. NIH had asked for $3.22 billion.

Memorial to Birmingham smallpox professor: Birmingham University is to set up a memorial fund to the late Professor Henry Bedson, who committed suicide earlier this year after Janet Parker, a medical photographer, died of smallpox virus which probably came from Bedson's laboratory. Professor Owen Lyndon Wade, Dean of the Faculty of Medicine and Dentistry at Birmingham, proposed the fund, and he hopes that it will provide money for junior staff at the university to travel abroad and undertake research, especially in virology.

In a statement, Professor Wade says that the scholarships would reflect Henry Bedson's care for the underprivileged in the third world. "Henry Bedson was a deeply conscientious man, constantly aware of the humane aspects of his work. His service to the World Health Organisation in the smallpox eradication campaign was outstanding, both in the laboratory and in remote areas of Pakistan and Afghanistan," he said. All who wish to give support to the fund are asked to send their contributions to the Dean's office at Birmingham University Medical School.



'Refusnik' refused: Viktor Brailovskii (right), who in 1977 succeeded Mark Azbel' as organiser of the Sunday seminar for Jewish refusnik scientists, has now been told by the tax authorities that he can no longer give private lessons in mathematics—his sole source of income since he was dismissed from a senior post as a computer scientist in 1972. The tax department said that Brailovskii could not give such lessons without a teacher's licence. No licence was issued, and Brailovskii could therefore at any time face a prison sentence for "parasitism"—i.e., being without visible approved means of support.



Meanwhile, his mathematician wife Irina (right), has received complete security clearance from the Rector of Moscow University, Anatolii Luganov. However, although the chief emigration office of the USSR has accepted the fact of her clearance, she was informed that this made no difference and she would still not be permitted to emigrate.

West Germany to build fast breeder reactor: Chancellor Helmut Schmidt's plan to build a fast breeder nuclear reactor at Kalkar, near the Dutch border, has been approved by the Bundestag, West Germany's lower House of Parliament. The plan was approved in a close vote of 230 to 225, but only after six members of the Free Democratic Party, which is in a coalition with the Social Democrats, finally agreed to abstain, after threatening to vote against the project.

The vote was crucial to Chancellor Schmidt because he believes that nuclear power is vital to West Germany's economic future. However, he faces strong opposition among the members of his Social Democratic Party, especially since last year when the Party chose coal as the chief source of energy in the future and called for a six-year moratorium on building nuclear plants.

Upjohn scientists induce bacteria to produce chick albumin: Scientists at the Upjohn Co., the US-based pharmaceutical manufacturer, claim to have used recombinant DNA techniques to induce bacteria to produce chicken albumin, a protein considerably larger and more complex than previous molecules produced in the same way.

Reporting their results in the Proceedings of the *National Academy of Sciences*, the scientists say that by transplanting a particular gene into bacteria, about 1.5 per cent of the bacteria's protein production becomes chicken albumin, and they expect that this level can be increased to 10 per cent.

According to the company, the experiment lays the groundwork for producing medically-useful quantities of enzymes and hormones, such as the use of bacteria to make the human blood-clotting factor missing from the blood of haemophiliacs.

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A refuge for scientists' writings

Professor Margaret Gowing, Honorary Director of the Contemporary Scientific Archives Centre, Oxford, explains how the centre was set up and outlines its work

History is essential to society and science and technology are crucial ingredients of history. History depends on records and therefore scientific and technological records should be systematically preserved. Until recently however records of 20th century scientists and engineers attracted much less interest than those of, say, politicians or novelists.

Most scientists and engineers work in institutions—government establishments, industrial firms or universities—and if all these institutions systematically gathered and retained records the residual problem would be small. But many of them do not. Thus, few British universities have archives staff and fewer still gather the papers of academics as they retire or die.

For these reasons, the Royal Society established a Committee on Scientific and Technological Records in 1967, under the chairmanship first of Sir Harold Hartley and then of Professor Nicholas Kurti. In 1969 a conference called by the committee agreed that scientists' and engineers' papers required special action, partly because general archives tended to be frightened by them. It was further agreed to establish not a special archive but a centre which would locate, sort and catalogue the papers of individual eminent scientists and engineers. Each collection would then be permanently deposited in an appropriate existing library and only the catalogues, reproduced by the Royal Commission on Historical Manuscripts, would be kept centrally and in the copyright libraries. The Wolfson Foundation gave a grant for an experimental period and I was asked to establish a centre at Oxford, where I had just been appointed Professor of the History of Science.

The Contemporary Scientific Archives Centre started in one basement room in April 1973 and has had a total paid staff of two people who do everything from typing, stamp-licking and driving to collect papers, to producing scholarly catalogues and orderly boxed collections, often from extensive and chaotic manuscripts. By the end of August 1978 60 collections had been handed over to 29 different libraries and a further 14 collections were in hand. In addition 175 enquiries had been made. The list at the end shows the variety of disciplines covered.

The collections cover scientists (including medical scientists) and engineers who died after the Second World War. The danger point for dispersal of papers is usually retirement but because of shortage of resources the centre can only deal with a very few collections of people as they retire. The Royal Society Committee decides who shall be included but the list is not confined to Fellows of the Royal Society nor do all Fellows automatically qualify.

By 'papers' the centre does not mean only scientific publications. The word includes papers of all kinds such as notebooks, notes of lectures heard or delivered, papers and correspondence covering all stages and all aspects of a scientist's life from school reports to political activity. The collections vary greatly in size and context. Some scientists keep few papers and some papers regrettably disappear: for example, the contents of the filing cabinets of Nobel prizewinner Cecil Powell, the physicist, were shredded soon after his death. By contrast some collections are very large, cover many years and are of outstanding interest: for example the papers of physicist Rudolf Peierls (including a life-long correspondence with Neils Bohr and Hans Bethe); Charles Coulson (including, besides papers on chemistry and mathematics, his many concerns such as pacifism, Oxfam and Christianity) or Patrick Blackett, the physicist (including his scientific, wartime, Labour Party and overseas activities). Some collections are beginning to form a 'critical mass' for the history of a discipline—for example in computing science.

The formula for the centre has proved very cost-effective, while Oxford University has helped greatly with administrative services and accommodation. Location in Oxford has also brought unstinting help and advice from many individuals. Nevertheless finance has been a constant anxiety, for the initial grants from the Wolfson Foundation and other funds were pump primers. At present the Royal Society and the British Library share the main cost with a smaller grant from the Council of Engineering Institutions. For two years from 1979 the Wellcome Trust will replace the British Library grant.

The centre has blazed a trail. Neither of the two staff members had any previous archival or scientific training but their work has been outstanding in quality and quantity and has attracted considerable interest abroad, especially in North America. The Wellcome Trust is now itself establishing a project on similar lines for medical practitioners and medical-related scientists. The work is becoming more valuable as more professional groups—such as various sections of physicists, molecular biologists, and computer scientists—become increasingly interested in the recent history of their disciplines and as more historians study not only the purely scientific work but the other public work and private interests of scientists and engineers. ☐

For further information and copies of Progress Reports (April and October annually) please write to Mrs Alton, Deputy Director, CSAC, 10 Keble Road, Oxford.

Collections completed and *in hand

Physics and Theoretical Physics
*Sir Edward Appleton, *L. F. Bates, *Lord Blackett, *H. A. H. Boot, S. Chapman, W. Cochrane, H. T. Flint, H. Heywood, H. London, Sir Rudolf Peierls, C. F. Powell, A. P. Rowe, Sir Frank Smith, E. C. Stoner, Sir Geoffrey Taylor, S. Tolansky, Sir John Townsend, R. Whiddington

Chemistry and Theoretical Chemistry
C. A. Coulson, Sir Cyril Hinshelwood, Sir John Lennard-Jones, Sir Patrick Linstead, *Sir Eric Rideal, *Sir Robert Robinson, N. V. Sidgwick, F. Soddy

Mathematics (including Mathematics of Computation)
British Association Mathematical Tables Committee, T. M. Chaundy, B. Egerton, S. Gill, D. R. Hartree, *C. Strachey, J. H. C. Thompson, A. E. Titchmarsh, A. M. Turing, F. J. W. Whipple

Biological and Zoological Sciences
W. T. Astbury, *J. R. Baker, D. Cunningham, H. M. Fox, Sir James Gray, E. J. Hanson, H. Hewer, E. Hindle, D. Keilin, D. Lack, Sir Wilfred Le Gros Clark, R. E. Moreau, *A. G. Ogston, *T. Weis-Fogh, *D. D. Woods

Medical Sciences
Sir Frederick Bartlett, Sir Roy Cameron, J. Cunningham, I. de Burgh Daly, N. M. Dott, Sir Wilfred Fish, G. W. Harris, D. Kirby, Sir Charles Lovatt Evans, R. Stacey, Sir Francis Walshe

Engineering
Sir Hugh Beaver, Lord Jackson, Sir Leonard Owen, E. A. Watson, *C. A. J. Young

Botanical and Agricultural Sciences
Sir Frederick Bawden, H. Hunter, W. Stiles

Geography and Geology
H. J. Fleure, *R. G. S. Hudson

Metallurgy
W. Hume-Rothery

History of Science
H. T. Pledge

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Environmental research in Northern Finland

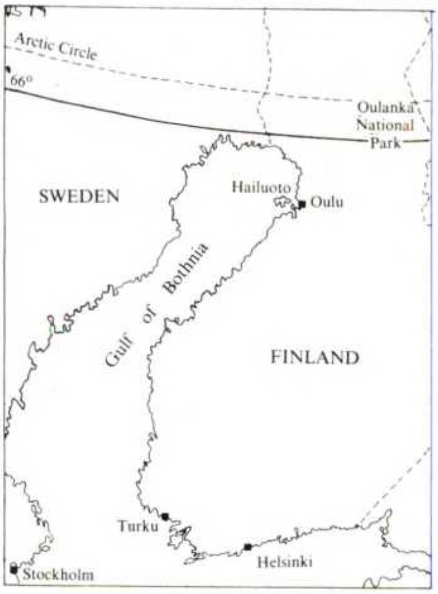
Peter D. Moore, plant scientist at King's College, London, reports from Finland.

JUST 150 km south of the Arctic Circle, Oulu in Finland is one of the most northerly university cities in the world. It lies on the Bothnian Bay, the northernmost spur of the Baltic Sea, and it has provided a centre for a variety of environmental research programmes in recent years.

The Bothnian Bay is covered by ice for between 150 to 200 days of the year, often to a depth of 4 m and has a salinity in its epilimnion of less than 0.2%. It is an unusual aquatic system. Oulu is an industrial city with a population of almost 94,000, so sewage and industrial effluent is a matter for concern. Research conducted by the University of Oulu at the Krunnit and Perämeri Research Stations on islands in the Bothnian Bay, have revealed that levels of inorganic nitrogen and phosphorus in the waters around Oulu are twice as high ($200–250\ \mu g\ l^{-1}$) as those 40 km north. The annual primary productivity is about $32\ g m^{-2}$ in the eutrophicated waters; this is twice as great as that found in the purer waters, but it is nevertheless only about a third of that found in the southern Baltic.

The low annual productivity can in part be accounted for by the prolonged frozen period, but even when liquid, the discolouring effect of humus colloids in the waters of the Bothnian Bay result in poor light penetration. The maximum depth for 1% penetration of light in summer is only 8–9 m.

The islands in the Bothnian Bay are rich in plant and animal life. On the island of Hailuoto the elk reaches its highest population density in Finland, about one elk per square km. Lichens and macro-fungi on the island have been extensively exploited. Of the lichens, *Cladonia alpestris* is valuable in horticulture and since it forms dense carpets beneath the pine forests which cover the dunes of Hailuoto, harvesting by hand is relatively simple. Up to 20% of the cover is removed. It is then left for five years to regrow, the growth rate being about $4–6\ mm\ yr^{-1}$. About 800 metric tons are harvested in Finland annually. The island of Hailuoto is also one of the richest sources of fungi in northern Finland. Estimates place the annual fresh weight yield at $200\ kg\ ha^{-1}$. Yields further north and east in Finland fall off to around $50–60\ kg\ ha^{-1}$, but in the south west they can reach as high as $260\ kg\ ha^{-1}$.



Oulu University has a field station on the west coast of Hailuoto from which survey work is conducted and it has a second research station in the east of Finland in the Oulanka National Park, just outside the Arctic Circle. Here the dense spruce forests with thick layers of moss were used as a site for intensive study in the International Biological Programme by Dr Paavo Havas.

The area is of particular interest to botanists because of the presence of calcium containing metamorphic rocks which permit the development of calcicolous plants such as *Cypripedium calceolarum* and *Kobresia simpliciuscula*. It may be the abundance of cations in local mires which has led to the survival in one inland spot of a population of *Triglochin maritima*, which is normally a coastal species. The existence of such a maritime plant in the Oulanka park led plant geographers at Oulu to speculate about the existence of a sea link between the Baltic and the White Sea when the area was depressed by glacial ice. Dr A. Vasari has been able to trace macroscopic subfossil remains of *Triglochin maritima* in the peats of its current locality going back 8,000 years. On the other hand, there are a number of maritime species, for example *Armeria maritima* and *Plantago maritima*, which have inland (often montane) ecotypes which were widespread during the closing stages of glaciation. *Triglochin maritima* could be another species of that type.

Finnish Lapland can boast yet another research station, this time belonging to the University of Turku, at Kevo in the far north of the country. It lies some 60 km north of the continuous pine forest in sub-arctic birch woods and fjell tundra. In this area, approaching 70°N, permanent ice cores are found within mounds, termed palsas, which typify the peatland habitats.

Much speculation has surrounded these palsa mires, in particular the circumstances under which they were created. Some people, such as M. Salmi (Turku) believe that all palsas began their formation at specific times in the past. He claims that the Lapland palsas fall into two groups which were initiated about 5,000 and 3,000 years ago respectively. Palsa studies in Ontario, Canada, by J. B. Railton and J. H. Sparling (Toronto), however, indicate a continuous and non-phased cycle of palsa initiation, growth and erosion.

M. Seppälä, working at the Kevo Subarctic Research Institute has introduced a novel, experimental technique into the discussion. His observations led him to believe that palsa mounds

Left : a patch of Cladonia alpestris; *right : growing palsa hummock*

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are created as a result of a failure of snow to accumulate on certain parts of the bog surface, so allowing ice penetration of those parts to be deeper. In winter, the wind blows the snow unevenly over the mire and, when temperatures fall to less than −40 °C, frost may penetrate to depths of 1 m.

If this explanation is correct, then it should be possible to create palsas experimentally by regular snow removal. Seppälä has contrived such an experiment and has kept experimental plots clear of snow during the past two winters. In the first winter, frost penetrated to 85 cm in the cleared area and only to 45 cm in the control. By mid August the control was ice free but the experimental plot was still frozen to a depth of 45 cm. In the second winter the artificial palsa had an ice core 100 cm deep and ice was still surviving to about 60 cm this September. The expansion of the ice core has now led to the surface of the mire in the cleared area being elevated 20 cm above its surroundings. This in turn has resulted in a natural wind clearance of the winter snow leading to more rapid palsa growth.

Studies such as these, together with lichen productivity, reindeer ecology and arctic nutrient cycling processes, all being carried out in Lapland's research stations, will undoubtedly contribute much to our lamentably poor understanding of the ecosystems of the far north. ☐

Down in a Polish forest, something is stirring . . .

SOUTHERN Poland, from an ecologist's point of view, could well be a nightmare—a jumble of conurbations, industrial enterprises and national parks, stretching in a wide belt across the country, while the high Sudeten-Carpathian mountain barrier effectively inhibits north-south ventilation. Not surprisingly, the forests of the area have deteriorated, and research into their management has been ranked as a "key" project.

In 1976, a detailed survey was begun into the management of these forests, with the participation of thirteen research institutions—four from the Polish Academy of Sciences, seven from the Jagellonian University and other academic institutions in Krakow, and two non-academic institutions (the Institute of Meteorology and Water Management and the Institute of Environmental Protection.) Some 70 senior research workers are involved in the project, ranging from ecologists and dendrologists to geochemists and economists.

The area chosen for study was the Niepolomice forest, some 30 km east of Krakow. The forest consists of two sections, a northern, deciduous part (~ 3,000 ha) and a southern coniferous part (~ 8,000 ha) mostly pine. Conifers are the most susceptible to pollution.

The forest, formerly a royal hunting park, is of considerable historical and touristic significance. It is ecologically unfortunate, therefore, that in the late 1940s, the new industrial town of Nowa Huta, with a steel mill still the largest in Poland, was established between Krakow and the Niepolomice forest. The prevailing wind is from the west, so that the forest receives the major proportion of industrial emission from Nowa Huta.

The siting of Nowa Huta was apparently primarily a politico-demographic decision, at a time when large-scale enterprises were seen to be the answer to the problem of rebuilding a war-shattered economy, at a time when no government anywhere was more than dimly aware of the concept of ecology as a planning factor.

After 25 years, however, the Niepolomice Forest was unhealthy, and a five-year project was undertaken, not only to find a remedy for Niepolomice, but also to establish a detailed mathematical model of the forest eco-system, and to propose methods of forest management in urban environments which could be extended to similar systems elsewhere.

On the basis of this computer model, a number of parameters were selected for field study, including dust-fall, wet and dry deposition of sulphur, heavy metal contamination, acidification of tree bark, loss of photosynthetic efficiency of pine-needles, energy flow via small consumers and predators, and the quality of roe-deer antlers. The situation was found to be far from promising: dust fall 45—80 tonnes/km^2-yr, wet and dry sulphur deposition 3·6—6·4 tonnes/km^2-yr, potential photosynthetic activity of pine-needles decreased by 13—18%. The total energy flow diagram revealed a slowing down of decomposition, producing a "carpet effect" under the trees, since the pine litter takes up to 10 years to break down completely, instead of the usual two, apparently due to inhibition of microbial activity, posibly due to heavy metal pollution.

Recent introduction of electrostatic precipitators in the Nowa Huta factories is improving the dust situation to a certain extent, but the problem of wet and dry sulphur deposition remains, so far, virtually insoluble. One proposal made by the research team is aerial spraying of the forest with lime, neutralising the sulphorous and sulphuric acid. This is being introduced into the nutrient spraying programme which has been carried out over the last three years and which is already proving a palliative—the timber increment has increased somewhat and the overall condition of the pines is improving. (Unfortunately, there is also the side effect of increased weed-growth and the eutrophication of the small river Drivnka).

In addition to this spraying, the team have put forward several other draft proposals. The forest stands, they say, should be rebuilt into a mixed forest with the possible introduction of exotic species such as black pine and Japanese larch at the western edge. (It is hoped in the course of the next ten years to determine the most resistant strains). The water-table should be raised by regulating the drainage channels in order to provide more available nutrients for the roots. Wild-life management (roe deer, red deer, wild boar), should be optimised, and steps should be taken to reduce tourist pressure by organising 'nature trails' on the British model.

A number of major problems still, of course, remain. For example, the net sulphur intake of the total biomass is still ~2 kg/year and the well-being of the forest can only finally be ensured by the radical control of industrial emissions. Nevertheless, the valuable contributions of the multidisciplinary team towards containing this problem is already effecting some amelioration, and at the same time has produced a valuable spin-off in the form of a number of published papers, and a book now in production, dealing with basic concepts of forest ecology and management.

Vera Rich

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correspondence

Piltdown hoax: new light

SIR,—If L. B. Halstead (2 November, page 11) had taken the trouble to look a little more closely at the evidence presented in the scientific papers on the Piltdown forgery (as well as in my book, *The Piltdown Hoax*) he would surely have realised (and this goes also for Mr Richard L. E. Ford) that the late Professor Douglas' arraignment of Professor Sollas is really without any foundation. The "evidence" of Sollas as instigator and co-conspirator with Charles Dawson amounts to very little and that, quite trivial and unconvincing.

Dr Oakley has already, in his letter to *The Times* (7 November 1978) shown that Douglas was mistaken in thinking that the Mastodon bones he said he gave Sollas were part of the Piltdown assemblage. The receipt by Sollas, according to Douglas' recollection of 68 years ago of a packet of potassium bichromate can hardly be taken as positive evidence for the reasons which Dr Oakley gives in his letter.

Moreover, Douglas should have known that Dawson had admitted to the use of bichromate, that Woodward knew this, and two people told me spontaneously (that is before the disclosure of the hoax) that they were aware of Dawson's staining activities. Moreover, as Oakley and I have shown, Dawson used a number of different methods of staining and we are left to suppose (on no evidence whatever) that Sollas initiated Dawson in all these methods.

The third item furnished by Douglas in his remembrance of Sollas borrowing apes teeth from the Anatomy Department at Oxford. If Douglas had remembered Sollas borrowing an ape's jaw or even better, an orang's jaw, that would have been rather more interesting, because it was an orang jaw with its molars that was used for the plants at the two Piltdown sites. To say that Dawson needed Sollas as a supplier is sheer guess work and unnecessary at that.

A likely source, as I have pointed out, was Abbott of Hastings, not to mention local museums and even straightforward purchase. A fourth item of "evidence" is the Sherbourne horse's head. For Douglas to say that this little curiosity (which cannot in fact be brought home to Sollas) is "an almost similar act" to that of the elaborate Piltdown Forgery is quite fatuous.

The motive attributed to Sollas for the humiliation of Smith-Woodward does not make sense. If his plan was to "expose" Smith-Woodward's gullibility and incompetence, why should he have so firmly supported the new *Eoanthropus dawsoni* in the first place? Why did he not wreak his vengeance by exposing the forgery at any time after Dawson's death in 1916? Halstead argues disingenuously that once Dart had discovered *Australopithecus* Sollas would have known that it was only a matter of time before Piltdown Man was exposed. But if Sollas was so sure about the status of *Australopithecus africanus* in relation to Piltdown, surely Dart's discovery provided the perfect opportunity (from 1925 onwards) to expose both Piltdown and Smith-Woodward.

The truth is that none of the leading palaeontologists of the day, including Dart and Broom (despite their many australopithecine discoveries), nor Leakey, nor Le Gros Clark, could see that Australopithecus made Piltdown Man impossible and (*ergo*) fraudulent. The reasons for this myopia, I have discussed elsewhere. A hint from Sollas or later Douglas, to any one of these would have been sufficient. Halstead is simply arguing from hindsight in suggesting that Sollas went to his grave rubbing his hands in anticipatory glee.

In my book I point out in some detail that a "mastermind" to guide Dawson through all the years of the complicated sequence of the events at Barkham Manor and Sheffield Park would need to be of Mephistophelean calibre. It would be tedious to repeat here all that is implied in asserting, or postulating, a continuous connection between Dawson and Sollas. Douglas and his sponsors do not even begin to face up to this. In the case of Sollas it would be quite as difficult a task as that which faced the recent accuser of Elliott Smith. Messrs. Halstead and Ford might care to look for proof of Sollas' alleged activities in the light of the arguments in my paper on Elliott Smith and Piltdown (*Symp. Zool. Soc. Lond.* no. 33, 23–26; 1973).

One thing the late Professor Douglas has succeeded in doing. He has certainly added a mystery of his own devising to the Piltdown saga—why should Douglas on such incredibly weak evidence take the trouble to besmirch Sollas' reputation?

Yours faithfully,
J. S. WEINER

The Athenaeum,
Pall Mall,
London

Proliferation

SIR,—In your article 'Is Proliferation Unstoppable' (26 October, page 681), you say that after the May 1975 Review Conference of the Treaty on the Non-Proliferation of Nuclear Weapons ". . . no outsider proceeded to sign". You cite this as evidence that "the NPT could hardly be said to be an unqualified success".

The facts are that during and after the conference eleven additional states joined the NPT. The new states included Libya, Venezuela, Japan, Switzerland and Portugal. A growth of 14%, including some important states, is not bad going. The treaty now has 104 members and Indonesia and Turkey are in the process of ratification.

Only 13 non-nuclear weapon states that have any significant nuclear plant remain outside the treaty; they include several of those mentioned in your article. However, in eight of these 13 every nuclear plant of which we are aware is under safeguards pursuant to non-NPT arrangements (like the German/Brazilian agreement). Only five non-nuclear weapon states have unsafeguarded plant—these include India, Israel and South Africa.

We agree that everything should be done to strengthen the non-proliferation regime, but one should not denigrate the considerable success of the treaty. A few years ago, German and Japanese ratification was by no means certain; now almost every leading industrial non-nuclear weapon state is a party. Moreover, IAEA safeguards have become much more effective and stringent in recent years.

Yours faithfully,
HANS-FRIEDRICH MEYER

IAEA, Vienna, Austria.

Responsible statements

SIR,—Though *Nature* is to be applauded for welcoming presentation of alternative views on controversial issues, I would think that the nature of the journal requires the editors to hold authors to a reasonable standard of responsibility, even on issues that evoke ideological conflicts. I therefore wonder why you lend the dignity of your pages to such statements as the following, in an article by Jonathan King (2 November, page 7): "Now we don't have a law in the United States that keeps anybody from introducing any gene into an epidemic strain of *E. coli* or *salmonella* or *Shigella*; and there are forces in that direction because of patents. If somebody else has a patent on making insulin in *E. coli* and you recognise that market what do you do?"

Yours faithfully,
BERNARD D. DAVIS

Harvard Medical School,
Boston, Ma., USA

Helpful oil companies

SIR,—The article on wave energy (30 November, page 433), gives a poor impression of the helpfulness of the oil companies, which I would like to correct.

UK OOA wave data is commercial and confidential, and there was a time when Wavepower Limited could not have access to it. However, that access was cleared some time ago via the Department of Energy—I was misinformed, and misled your reporter.

Nevertheless you misrepresent both the oil companies and myself when you say that I have been unable even to arrange a visit to one of the platforms. My enquiry at the conference was the first time of asking, and I have already had two offers in reply.

Yours faithfully,
M. J. PLATTS

Wavepower Limited,
Southampton, UK.

news and views

Spacecraft charging

from Gordon L. Wrenn

Halloween is traditionally the time when things go bump in the night and was somehow appropriate for a Spacecraft Charging Technology Conference sponsored jointly by the United States Air Force and NASA. Justification for the three day gathering of some 150 scientists and engineers really stems, not from physical bumps but from a host of status anomalies seen on operational spacecraft at high altitudes. The proposition is that these malfunctions, ranging from nuisance level to a possible fatality, are caused by electrostatic charging events which occur near local midnight when a satellite passes through the shadow of the Earth. Six sessions at the impressive Air Force Academy in Colorado Springs served up 54 well presented papers; these demonstrated the obvious interest and support that the field has attracted. Why, then did the Conference close with a panel discussion on the theme 'The Spacecraft Charging Hazard—is there a credibility gap?'; futhermore why was the only answer 'Yes'.

The electrostatic potential of any satellite will float to a value which produces zero nett current. At low altitudes, a high concentration of cold ionospheric plasma clamps the floating potential at a small negative value. Up at several Earth radii, outside the plasmasphere, the cold plasma decreases, the photoemission current due to sunlight becomes dominant and the spacecraft charges to a few volts positive. In eclipse negative charging is to be expected but the absence of cold protons could permit a potential build up to very high values—20 kV has been observed. The geosynchronous orbit (at 6.6 R_E) is a popular one for a variety of applications satellites but with eclipse times of up to 75 minutes and a location at which intense fluxes of high energy electrons are encountered during periods of geomagnetic disturbance, it seems to be particularly susceptible to charging. The problem is complicated by the multiplicity of surface materials in use. In the same way that there is little danger when lightning strikes an aircraft, overall charging is not a threat but the possibility of differential charging presents the real hazard; subsequent discharge could generate a large current spike on power or ground lines and affect sensitive solid state devices.

Since the last such conference in October 1976, much good work has been done on modelling both the particle environment and electrostatic fields around spacecraft. Considerable advances have been made in understanding surface material properties and developing active potential control systems utilising electron or ion beam emitters. Studies are now well in hand for huge space platforms such as solar power satellites with areas over 100 km^2, and charging characteristics will clearly be of utmost importance to their design. One outcome of the earlier conference was a resolution to include simple discharge monitors on all operational spacecraft but this has been largely ignored and the study of anomalies has progressed little. Satellite designers have not been convinced that the hazard is serious enough to justify increased production costs.

The scepticism is bred by a number of missing links in the whole discussion. A considerable catalogue of anomalies does have some correlation with geomagnetic activity but generally does not show an expected pattern with local time. Evidence for kilovolt charging comes exclusively from the ATS 5 and ATS 6 satellites but even these exhibited no corresponding status anomalies. There have been many other suitably instrumented satellites for which charging events have remained absent or undetected. Laboratory simulation of the charging process is difficult and has not yet been achieved on a system level. NASA and USAF hope to obtain all the answers with the SCATHA satellite which is due to be launched in January 1979; it promises a concerted attack on all the factors which make up the charging equation. Whilst it necessarily represents a compromise between the desire to create charging problems for study and the need to pursue techniques which might overcome these same problems, it should mark a big step forward on the trail of missing links.

ESA, the European Space Agency, is in a strong position to contribute to the subject. In addition to having a good background in the physics of space materials, it has, in orbit, OTS and METEOSAT spacecraft with the opportunity for careful monitoring of anomalies and it has GEOS 2. In contrast to ATS, the electromagnetic cleanliness of GEOS is excellent. Over 96% of the surface has been made conducting by the use of special paints and an indium oxide coating for the solar array. Differential charging has been avoided but satisfactory thermal characteristics have been achieved and for the first time reliable cold plasma measurements are available at 6.6 R_E. So far no significant charging events (in excess of about 150 V) have been seen on GEOS and it may be that its overall susceptibility is very different from that of ATS. If this is true, it is probably due to different secondary emission properties of the resulting retarded impact energy, the secondary electron yield exceeds one. Selection of suitable secondary emitting surfaces could provide an inexpensive method of limiting the extent of charging.

It is planned that GEOS and SCATHA experimenters should collaborate closely on data interpretation and this presents the best chance of closing any credibility gaps. As to the real significance of the whole issue, it

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could be that the approaching solar maximum will provide the opportunity for everyone to judge; our lives are now much more dependent upon synchronous satellites than they were 11 years ago. ☐

Membranes and parasites

from D. F. H. Wallach

A WORKSHOP on the membrane pathobiology of tropical diseases held recently* brought together experts in the various diseases and membrane biologists, biophysicists, biochemists and immunologists to discuss ideas and techniques concerning membrane structure and physiology in relation to problems of parasite/host interactions.

It became evident very early in the meeting that membrane biology represents a major area of common ground for all the major parasitic diseases. Membrane studies are relevant to the modes of action of antiparasitic drugs, and membrane-bound antigens may be of particular importance in the development of serodiagnosis and immunoprophylaxis.

Intracellular parasites, such as the plasmodia which cause malaria, interact with the host cell membrane during entry and exit from the cell, and may modify the membrane during their intracellular development. These different processes may be subject to interference by immunological or chemotherapeutic methods. At least three organisms of those under consideration, *Mycobacterium leprae, Leishmania* and S. American trypanosomes, are taken up primarily by macrophages by phagocytosis, but have evolved mechanisms by which they evade the normal destruction of foreign material within phagolysosomes. These organisms may also avoid immunological defence mechanisms through their sequestered intracellular location.

The presentations on extracellular parasites revealed additional mechanisms which help them to evade host immune defences. African trypanosomes have the capacity for extensive variation of the chemical structure and antigenic characteristics of a glycoprotein surface coat lying outside the plasma membrane, and invading schistosomes have the remarkable ability to incorporate into their surface membranes a variety of host plasma membrane components, including blood group substances and components of major histocompatibility antigens, mimicking host surface membrane antigenicity, and to shed, at least *in vitro*, surface membrane material in a manner reminiscent of the behaviour of some tumour cells.

The workshop revealed that, although extensive immunological information exists on changes in the plasma membranes of host cells and the surface coats or membranes of pathogens during the processes of infection, evidence on the biochemical, biophysical, physiological, genetical and micromorphological processes involved is meagre or totally absent. However, recent developments now permit these approaches to studies of parasite infection. For example a major causative agent of human malaria, *Pl. falciparum*, can now be cultivated continuously *in vitro*, the technique has been established in several laboratories around the world and its expansion to mass-cultivation seems feasible. The major steps in the fractionation of host cell (erythrocyte) and parasite antigens in a model monkey malaria have been achieved; parasite modification of host cell membranes can be analysed both biochemically and immunochemically and the system allows rigorous biochemical analysis of parasite, parasite-induced and host-cell-modified membrane lipids and proteins, as well as numerous other metabolic variables. Although it has not yet been possible to cultivate *M. leprae in vitro*, the organism has been found to grow well in the liver and spleen of most forms of nine-banded armadillos. This permits production of large amounts of organisms free from host cell material, and studies are in progress which should eventually lead to vaccination and improved chemotherapy. Studies of the amastigote forms of leishmania growing within macrophages have revealed that some forms of leishmania modify the surface of host cells in a fashion which is recognisable immunologically. Some forms of trypanosomes can be cultivated either in the absence of cells, or using host cells only as 'feeder' cells. Some forms of S. American trypanosomes can now be cultivated on a scale adequate to permit isolation of mutants, setting the stage for genetic experimentation. Moreover, fractionation and biochemical studies, involving both surface and cytoplasmic membranes, have been initiated using these organisms, as well as leishmania.

Animal models exist for schistosomiasis and, although some 'conventional' biochemical/molecular biological approaches appear less readily applicable to this area, the discussions pointed to the existence of an important group of microtechniques, including micropuncture, microcatheterisation and X-ray microanalysis, which are immediately available to the resolution of critical areas in schistosomiasis. Many of these approaches appear equally relevant to filariasis.

The workshop showed that, in spite of promising beginnings in basic research on the six major diseases, there are important lacunae in information about both parasite and host-cell membranes, which might be filled by application of techniques for membrane studies which are now standard in many laboratories. Present knowledge on the relationships of intracellular parasites within the host cell raise many pertinent questions regarding transport mechanisms within the parasite/host cell complex, particularly of K^+, Na^+ and Ca^{2+} transport and the ionic preferences of intracellular compartments. These issues could be resolved by flux analyses, possibly combined with specific ionophores, and also by use of intracellular ion-localisation techniques. The effects of possible parasite-induced alterations of Ca^{2+}-fluxes deserve major attention. In another vein, the major anion-transport protein of erythrocyte membranes has been extensively studied, many of its active sites can be specifically labelled and application of these approaches to the membranes of erythrocytes parasitised by malarial parasites is practicable. Such labelling methods could also fruitfully be extended to macrophages (normal and infected) and to schistosomes.

Most participants felt that further progress in the understanding of the six diseases required basic research in the following areas.

Host and parasite surface receptors; parasite surface coats; passage of intracellular parasites into and out of cells fractionation of host-cell and parasite membranes; membrane composition of host cell and parasite; antigenic variation in parasite and parasite-induced antigens, and immune mechanisms; application of the hybridoma technique to the study of parasite antigens; transport and permeability; membrane enzymes; genetics; propagation of the pathogenic organisms involved; model systems.

Scientists interested in collaborating

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*A workshop entitled The Membrane Pathobiology of Tropical Diseases, sponsored by the Special Programme for Research and Training in Tropical Diseases of the World Health Organisation was held at Titisee, Federal Republic of Germany on 4–8 October, 1978.

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in these or other activities of the Special Programme should contact the Director, Special Programme for Research and Training in Tropical Diseases, World Health Organisation, Geneva, Switzerland, for further information.

Virus precursor shedding from mammary tumour cells

from A. J. McClelland

IN 1976 Ritzi *et al.* made the intriguing observation that mice with murine mammary tumour virus-induced mammary tumours had much greater amounts of the major mammary tumour virus (MuMTV) structural glycoprotein gp52 in their blood than did mice with no mammary tumours. This was true even when the tumour-free mice were productively infected with MuMTV and had very high levels of gp52 in their milk. Ritzi's work raised several interesting questions. How are the MuMTV glycoproteins synthesised and assembled, what is the mechanism and biological function of gp52 secretion by mammary tumour cells and are there differences in the synthesis and assembly of MuMTV in normal and transformed cells?

Thanks to the establishment of continuous MuMTV-producing mammary tumour cell lines (Ringold *et al. Virology* **65,** 135; 1975; Sarkar *et al. Virology* **77,** 12; 1977) some answers to the first question at least have been found. It appears that the MuMTV glycoproteins are derived from a single precursor molecule, designated pre-gp70 (molecular weight 70,000) which is then cleaved to form the glycoproteins used in virus assembly (Racevskis & Sarkar *J. Virol.* **25,** 374; 1978; Schochetman *et al. Virology* **85,** 168; 1978; Dickson & Atterwill *J. Virol.* **26,** 660; 1978). Even more interesting is the unexpected discovery that the MuMTV glycoprotein precursor is shed from the surface membrane of the tumour cells in which it is synthesised.

Racevskis and Sarkar's discovery, that both gp52 and its precursor, pre-gp70, were present in virus-free culture medium from MuMTV-producing cells was unexpected because it was the first time that a glycoprotein precursor had been found in cell culture medium and because cell surface labelling studies had shown that, unlike MuMTV gp52, pre-gp70 is not present on the surface of MuMTV-producing cells.

Since pre-gp70 is shed into the medium of MuMTV-producing tissue culture cells, yet is apparently not available for surface labelling, two populations of pre-gp70 may exist. One population, destined to provide mature proteins for MuMTV assembly, may be cleaved before reaching the cell surface to produce gp52. The gp52 is then inserted into the cell membrane and leaves the cell only when it is incorporated into a virus particle. The second population of pre-gp70 molecules are shed from the cell without ever becoming integrated into its surface membrane. In that context, it is interesting to note that pre-gp70 has not been reported to be packaged within mature MuMTV particles.

The anti-gp52 serum that Ritzi used (*Proc. natn. Acad. Sci. U.S.A.* **73,** 4190; 1976) reacts with both gp52 and pre-gp70 and thus the glycoprotein that was detected in the plasma of tumour-bearing mice might have consisted of gp52 alone, gp52 and pre-gp70 or even pre-gp70 alone. The discovery that pre-gp70 is shed from mammary tumour cells in tissue culture could therefore explain the observation of Ritzi *et al.* but the mechanism by which MuMTV glycoproteins are shed from cells and the biological significance of shedding are still unknown. However, one can reasonably speculate that shedding helps MuMTV-producing tumour cells to escape from the immunological defences of the host. Immunisation studies have shown that gp52 is the most potent immunogen for protecting mice from exogenous MuMTV infection and subsequent tumorigenesis (Sarkar & Moore *Cancer Res.* **38,** 1468; 1978). It is therefore possible that the release of large quantities of gp52, and possibly pre-gp70, into the plasma provides a means of complexing (and thus, in effect, neutralising) cytotoxic antibodies against the glycoprotein, thereby increasing the survival advantage of MuMTV-producing tumour cells.

Whatever the biological function of glycoprotein shedding, it provides a unique opportunity to investigate two apparently different pathways of pre-gp70 transport and/or processing. It is still not known if MuMTV-producing normal mammary gland cells cultured *in vitro* would shed viral glycoproteins. If it is found that such normal cells do not exhibit the shedding phenomenon, membrane changes taking place in the transition from normal to tumour cells might be studied using MuMTV glycoprotein shedding as a marker for cell transformation. □

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Versatile accelerators

from R. G. P. Voss

THE range of uses to which small accelerators are now put is astonishing. These were covered exhaustively in a recent conference in Texas on the application of small accelerators*. The word 'small' is perhaps misleading: there were progress reports on the two very large tandems being built in the United Kingdom and in the United States, at Daresbury and Oak Ridge respectively, and experiments carried out on the 800 MeV proton linear accelerator at LAMPF and the 20 GeV electron linear accelerator at SLAC were reported. Perhaps, as someone remarked, even small accelerators are big in Texas.

Amongst the many different fields, one topic inevitably attracted considerable interest: the application of accelerators to medical purposes. Several papers dealt with the production of radioisotopes by cyclotrons, tandems, and linear accelerators. Information was given on targets, yields, the subsequent radiochemistry, the various uses of the isotopes, and instrumentation to detect localised radioactivity in the human body. Considerable work has been done in recent years at Los Alamos with pions produced by the proton linear accelerator. This is a huge machine, 850 m long, and its suitability for widespread use in medical work is obviously restricted. As a consequence a smaller accelerator, a mere 120 m long, is now being developed. It is known as PIGMI (Pion Generator for Medical Irradiations) and is now in its third year of development, with emphasis on being less expensive ($10M) and more reliable than accelerators for pure research.

A related field of great interest is that of computed tomography, and developments following from the original EMI CT scanner were presented. Work on proton CT at Los Alamos was described by W. F. Hanson (Oak Ridge Associated Universities) and R. R. Highfill and M. E. Phelps (University of California at Los Angeles) gave a paper on positron tomography. By detecting the annihilation radiation from positrons one can make an *in vivo* study of physiological processes in specific regions of the body. The need for positron-emitting radioisotopes (such as ^{11}C, ^{13}N, ^{15}O, ^{18}F) with short half-lives means that

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*5th Conference on the application of small accelerators, North Texas State University, Denton, Texas, 6–8 November 1978. Abstracts are published in the Bulletin of the American Physical Society, volume 23, number 8, 1978.

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accelerators must be available very close to the medical centres.

Two papers dealt with a subject of great topical interest. K. H. Purser (General Ionex Corporation) reviewed the use of electrostatic accelerators to give ultrasensitive particle identification, resulting in a spectrometer which is sensitive to a few tens of particles in a background of 10^{18}. By stripping electrons after an initial acceleration, one dissociates molecules and so enormously simplifies the identification of the atoms one is looking for. These need not be radioactive isotopes (as was necessary, for instance, in the previous method of counting ^{14}C decays): it is now possible for instance to study Li, Mg, Si and Cl. Moreover, the rare earths form excellent tracers. Extensions to the technique are leading to a new generation of secondary ion mass spectrometers many orders of magnitude more sensitive than earlier ones. H. E. Gove (University of Rochester) reviewed progress in ^{14}C dating in the remarkable 18 months since the technique of using an electrostatic accelerator was first reported. It is possible to date back to 70,000 years or even earlier, and a few milligrams of sample are sufficient (Doucas *et al.* *Nature* **276,** 253; 1978 and Hedges & Moore *Nature* **276,** 255; 1978). The technique is being constantly extended. Work has been done on ^{36}Cl for instance, for dating old ground water with a view to the safe storage of nuclear waste (as reported in this issue of *Nature* on page 22) and on ^{10}Be for 'million year geochronology'.

A number of papers dealt with various aspects of thermonuclear reactions. D. J. Nagel (US Naval Research Laboratory) discussed the progression from laser light through electrons to light ions and then to heavy ions in developments aimed at thermonuclear fusion. Currents of 300 kA of protons of energy about 1 MeV can be produced, with substantial fractions of a terawatt in a few nanoseconds. Even higher pulse energies are planned using 25 GeV uranium ions. Intense ion beams on solid targets will produce and heat plasmas to temperatures of the order of 10^5 K. He also described fascinating work on multi-atom projectiles: diamond dust is levitated while charge is built up with, for instance, a proton beam. Then the dust particle is accelerated by a 2 MV Van de Graaff accelerator. Twenty such particles can be accelerated per day! These are then fired into solid targets, to get plasmas with temperatures of the order of 10^4 K.

Work on new uses of polarised beams was presented by S. S. Hanna (Stanford University). A polarised beam is implanted in a target, and if this has the appropriate properties the polarisation transferred to the nuclei of the target can be preserved. This serves as a probe for studying magnetic moments, quadrupole moments (if an electric field is present), hyperfine fields, lattice effects, and for solid state problems such as the relaxation time of an implanted nucleus in a holding material. Polarised beams are being used as gas targets, thus enabling polarised-polarised reactions to be studied.

S. Raman (Oak Ridge National Laboratory) reported on a search for superheavy elements in giant-halo monazite inclusions. Use was made of the particular advantages of synchrotron radiation (from the electron storage ring SPEAR at Stanford) to induce fluorescence X rays. No sign of superheavies was detected.

Many other topics were covered: geoscience and mineral exploration, neutron activation (with applications to agriculture, archaeology, the life sciences), trace element analysis, radiation processing of foods and sterilisation of medical products, the curing of paint, the crosslinking of polymer insulating and packaging material. Papers on ion implantation covered amongst other things the processing of semiconductors, integrated circuits, metal corrosion, and a project for an implantation for silicon solar cell processing (P. H. Rose, Nova Associates) with a capacity of about one acre per day!

The overall impression of the conference was the enormous and rapidly growing use of accelerators in an extraordinary range of applications. ☐

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Semliki forest virus: key to the MHC?

from Polly Matzinger

IMMUNOLOGISTS have long been intrigued and biochemists frustrated by the cluster of genes collectively known as the major histocompatibility complex (MHC). To immunologists it is a fascinating bit of chromosome. Cell surface molecules coded by genes in the MHC have a central role in the processes by which the immune system recognises self from non-self (see *News and Views* **272,** 112; **274,** 840; 1978;). They are involved in the recognition processes which lead to graft rejection, immunity against infectious agents, susceptibility to a wide range of diseases, and have even been shown to affect mating preferences in mice (*Immunogenetics* **6,** 265; 1978). Consequently, many theories and much of the current work in immunology concern the MHC.

The MHC has been difficult to study biochemically. Purification and characterisation of its products has been an arduous task replete with surprises. In 1956 the mouse MHC, H-2, was thought to be DNA. In 1957 it was a carbohydrate and by 1966 it had turned into a lipid. Today it is a glycoprotein with the arguments still raging over the antigenic importance of the carbohydrate parts (Parish *Immunogenetics* **6,** 343; 1978). A major cause of frustration and confusion has been the difficulty of purifying MHC products in sufficiently large quantities; however, a recent article from Helenius *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75,** 3846; 1978) promises an easier road ahead.

Ari Helenius and his colleagues report that Semliki Forest Virus binds preferentially to MHC products on the cell surface. They discovered this while testing binding properties of the virus. They were using antibodies directed against Semliki Forest Virus to detect its binding to cells and antibodies directed against H-2 as a control. To their surprise they found that target cells which had been incubated with Semliki Forest Virus would no longer bind anti-H-2 antibodies. They postulated that the virus was binding to H-2 and therefore blocking the H-2 molecules from the anti-H-2 antibodies. Further tests showed that Semliki Forest Virus also bound to human cells bearing HLA molecules (the human MHC) but not to their mutant counterparts lacking HLA. Moreover, the virus binds to artificial lipid vesicles which have had MHC products inserted and can even be used on a Sepharose column to purify MHC molecules from solubilised membranes. So Semliki Forest Virus should make the purification of MHC molecules much simpler.

Like protein A from *Staphylococcus*, which is useful in the purification of antibody molecules, Semliki Forest Virus has another interesting use. Protein A, which binds to the Fc part of antibodies has been used in phylogenetic screens to study the evolution of antibodies (Kronval *et al. J. Immun.* **104,** 140; 1970). Semliki Forest Virus may do the same for the MHC. There

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has been much speculation about the evolution of the MHC. Simple self–non-self discrimination is found in very primitive organisms. Sponges only fuse with genetically similar sponges and, in colonial ascidians, recognition of the products of a single locus determines whether free swimming tadpoles will join up to form colonies. Even mating preference is controlled by this locus in ascidians. It has been postulated that the MHC may have evolved from genes that control such primitive self recognition and that our immunological recognition system is simply a more sophisticated version of that of amoebae and sponges (Langman *Rev. Physiol. Biochem Pharm.* **81**, 1; 1978; Lafferty & Woolnough, *Immun. Rev.* **35**, 231; 1977; Burnett & Fenner *The Production of Antibodies*, Cambridge University Press, 1949). So far such speculation has chiefly fuelled fireside debates, but now Semliki Forest Virus could be used to test these theories.

The virus has an amazingly wide host range, having been grown in insect as well as avian and mammalian cells. To infect such a wide array of cells it must either have a wide range of cell surface receptors or a receptor which is evolutionarily highly conserved. A hint that the receptor is conserved comes from Helenius *et al.*'s paper showing that the virus binds both human and mouse MHC molecules. Will it bind to primitive organisms? We may soon have an answer from Virginia Scofield and Irv Weissman at Stanford who are testing its binding to the colonial ascidian *Botryllus schlosseri.* Binding *per se* is no proof that the molecules are evolutionarily related, but Lee Hood's group at CalTech may have a solution to that problem. They will soon be able to sequence picomolar quantities of protein at the rate of 40–60 residues a day. It will then be entirely possible to isolate a Semliki Forest Virus binding protein from a small number of *Botryllus* (say one seaweed leaf's worth) and sequence it to see how it compares with the evolutionarily more advanced MHC molecules.

The story is told of an explorer who, coming on a forest not marked on his maps of Africa, asked his native guides what it was called. 'Semliki', they said, shaking their heads, 'Semliki'. Little did he know when he named it Semliki that Semliki means 'I don't know'. Ironically the virus named Semliki may well fill in some of the gaps in our knowledge of the elusive MHC. ☐

Storing energy as acid

from A. B. Hart

WHEN everybody had a woodpile, a coalshed or an oiltank there was little fuss about matching the rate of production of an energy source to the consumer's need. But for the consumer with a taste for the convenience and cleanness of electricity in his domestic or industrial premises it is another matter. Although electricity plants can lay in stocks of coal, oil or uranium the plants themselves are costly and complex and it is both financially and mechanically unsound to turn them down as the demand for electricity falls back in its diurnal or seasonal cycle. Indirect storage of domestic electricity is possible in off-peak space heating and in the future perhaps in electric battery motor cars. This kind of consumer 'storage' may well play a large part in the future planning of a power network, but there will remain a need for a balancing store near the generators and under the control of the operators of the network, so that the plant can be kept running at a steady load.

In recent years true storage has been available using hydroelectric plant in reverse; storing electrical energy as potential energy of water. But hydroelectric plants are usually expensive to build and in the UK there are going to be few sites left to meet the demand for storing night-time electricity expected around the turn of the century. This is when the proportion of electricity generated by nuclear power (not to mention wind and wave power with their unavoidably intermittent characteristics, which call for a very large storage element) is likely to be increasing rapidly.

Other methods of energy storage have recently attracted much interest. Flywheels, superconducting magnets, compressed air and heat, underground water reservoirs, and rechargeable electrochemical batteries are all under scrutiny (Gardner *et. al. CEGB Research* **2**, 1975). One of the more radical ideas is to make hydrogen from spare electricity by electrolysing water or directly from nuclear heat. The hydrogen need not be entirely reconverted to electricity; it can be used as a chemical feedstock—for fertilisers, petrochemicals and for hydrogenating coal to provide liquid fuels. This is an introduction to the much broader idea of the 'hydrogen economy' (Getoff *Wasserstoff als Energieträger*, Springer Verlag, Vienna 1978) a scenario in which all primary energy sources old and new, fossil, nuclear, solar, winds, waves and biomass, are converted into hydrogen which is stored as liquid or gas and piped to the consumer for his total energy neds. Objective attempts to make an economic case for such an all-embracing hydrogen energy system depend very much on the cost, efficiency and safety of storing the hydrogen. For fuelling transport one could use liquid hydrogen or a metallic hydride, neither of them cheap nor particularly attractive, the former because of the cost of the container, the latter because of the heavy weight of the metal. For storage in large-scale power networks one thinks of the gas in high pressurised pipe-lines or underground caverns. All feasible but somehow a little unnerving; and the feeling remains that storage and transport is rather a weak point in the idea of the hydrogen economy.

A recent paper from the RCA Laboratories at Princeton in the US (Williams, Crandall & Bloom *Appl. Phys.* **33(5)** 381; 1978) suggests an interesting alternative. Linking the hydrogen to CO_2 to give formic acid, HCOOH, from which it can be released using a palladium catalyst, one obtains a stable liquid energy storage medium, equivalent, even with the formic acid as a $4\ \mathrm{mol}^{-1}$ (4 M) aqueous solution, to the gas held at 100 atm pressure in a heavy cylinder. The acid can be made electrolytically in the 4 M solution by the reduction of CO_2 (or bicarbonate ions, HCO_3^-) with oxygen appearing at the other electrode. Using an amalgamated nickel electrode for the CO_2 reduction with an ion exchange membrane to separate it from the oxygen electrode the authors observe only an extremely small current ($1\ \mathrm{mA\ cm}^{-2}$) even with a high overpotential (1.0 V) denoting a very low electric energy to hydrogen conversion efficiency and a low specific output—which implies a very large investment in electrolysis plant. On such a basis the conversion cost would be prohibitive even without taking account of the palladium hydrogen release catalyst. And that is not cheap; a simple calculation suggests over £3,000 worth of palladium-carbon at current prices to liberate enough hydrogen to power a small motor car (3.5 kW electrical output). But such calculations are mischievous—the authors have made a laboratory demonstration of the process, not a pilot plant demonstration unit.

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It is the basic idea of going directly from electricity to a stable liquid energy storage medium which is so exciting. Further work must examine what energy losses are inevitable in the formic acid system to see whether they can be accepted and whether other losses can be avoided at acceptable cost. Electrochemical studies must also show whether by working under different conditions, for example, at higher temperatures and pressures, one could achieve a higher concentration of the acid (a 60% solution would contain more than half as much hydrogen on a volume basis as liquid hydrogen itself). It is by no means evident that the electrolytic liberation of hydrogen atoms in close proximity to CO_2 molecules, leading to the formation of HCOOH, should not at a suitable surface, proceed as efficiently at a given surface-specific rate as in the absence of CO_2. In this case the atoms form hydrogen molecules, as in the electrolysis of water to form hydrogen gas at the cathode. And advanced electrolysis of water can achieve a high (75–90%) efficiency of conversion of electrical energy into hydrogen (expressed as the heat of combustion to water), vastly higher than the authors' experiments seem to suggest for formic acid formation. A review of the electrochemical literature (Russell *et al. J. Electrochem. Soc.* **134,** 1329; 1978) suggests that the authors' low efficiencies and low specific rates of formic acid formation are expected for this type of electrode process, where the hydrogen atom must be liberated at a high over-voltage (also with very poor electrical efficiency) to be able to break open the CO_2 molecule and react with it. One might get over this problem but it is apparent that considerable research is needed.

Meanwhile, it may certainly be reasonable for the authors to claim, as they do, that the system "represents an energy storage method of exceptional versatility and generality" but until much more work is done it is surely rather over-optimistic to state that it "will be important for the storage of elecrical energy whenever there are surpluses". □

European plant health

from a Correspondent

THE introduction of plant pathogens into a country in which they are not normally present can have devastating economic or environmental consequences. To prevent the inadvertent introduction of serious pathogens, stringent and well-informed quarantine regulations are necessary. New quarantine measures for continental Europe and Britain have been proposed by the European and Mediterranean Plant Protection Organisation (*EPPO Bull*, special issue 1975) and the EPPO have recently published the first set of data sheets on which these recommendations are based (*EPPO Bull.* 8(2); 1978). These data sheets list some of the organisms covered by the quarantine regulations which are classified as A1 (organisms not present in the EPPO area) and A2 (present in the EPPO area). They provide a description of the pathogens and some of the diseases they cause.

At first glance this appears a commendable piece of work, but is it? One deficiency, which has caused widespread concern amongst plant pathologists who have the task of interpreting the regulations is that variations in strain, race and serotype are ignored. The potential importance of such differences can be seen in British hedgerows, now lined with rows of dead elms as the result of the introduction of a variant strain of a pathogen already classified as endemic within Europe (including Britain).

With regard to the diseases with a viral aetiology the picture is confused by the lack of any reference to a definitive description of the virus such as the AAB/Commonwealth Mycological Institute Descriptions of Plant Viruses, and by the omission of any description of the morphology, composition, grouping or serological grouping of the virus. How then is anyone to know exactly which virus is being referred to and in that case how can "an EPPO country take whatever action is necessary and reasonable to prevent introduction" (*EPPO Bull.* 1975 special issue).

Plant virus nomenclature is notoriously confused by the practice of naming the viruses after the disease they cause and it seems a shame that the EPPO have not learnt this lesson. For example they describe Scottish raspberry leaf curl as a 'completely different disease' from American raspberry leaf curl and attribute rose wilt syndromes to endemic A2 pathogen(s) when plainly this is unlikely to be the case.

In Britain and Holland no clear evidence that rose wilt or dieback was graft transmissible has been found (*Phytopath Z.* **79,** 160; 1974; *Neth. J. Pl. Path.* **81,** 187; 1975); whereas in New Zealand the pathogen was readily graft transmitted and some field spread has been recorded (*N.Z.J. agric. Res.* **14,** 735; 1971; *A. J. R. N. Z. Inst. Hort.* (2), 16; 1974). In California rose spring dwarf (*Pl. Dis. Reptr.* 55, 294; 1971) was reported and described as a graft transmissible disease distinct from rose wilt (*Pl. Dis. Reptr.* **60,** 183; 1976). In addition rose leaf curl, a distinct component of a disease complex which resembles rose wilt, has also been described in California (*Pl. Dis. Reptr.* **60,** 178; 1976). The question therefore remains of what is rose wilt and which pathogens cause it. Poor graft union formation, stunting and proliferation are disorders that can be induced by many pathogens, physiological conditions and environmental factors in woody plants. In view of this, to suggest endemic A2 pathogenic causes and to recommend quarantine procedures on that basis is both naive and foolhardy. Quarantine procedures should be based on the aetiology of a disease, not on disease expression.

The EPPO Working Party also seems to be rather inconsistent in its recommendations for the control of A1 pathogens. For the control of needle cast of Japanese larch caused by *Mycosphaerella larici-leptolepis* they state that "the importation of larch from Japan should be prohibited". However, in respect of American plum line pattern virus they say that it is doubtful whether roguing of trees should be recommended. This contradicts the original recommendations for A1 organisms of taking necessary and reasonable action to prevent entry.

The lists of A1 and A2 pathogenic organisms are abysmally short when one considers the vast numbers of economically important pathogens that have been described throughout the world. It is to be hoped that EPPO will rapidly extend their lists and that the data sheets will provide more comprehensive and accurate information on these pathogenic organisms.

Quarantine measures can only be effective if those responsible for them are well informed. Bureaucrats with little understanding of plant protection must seek the guidance of leading research workers otherwise confusion will result.

Non-endemic pests and pathogens are being imported to Britain annually, and the situation in continental Europe is even worse. EPPO's function is to advise on the restriction of movement of pathogenic organisms into and within Europe without impairing plant trade. Let us hope that it will adopt the correct policies to fufil this role and not imitate the Plant Health Branch of our own Ministry of Agriculture, Fisheries and Food (see *Nature* **259,** 449; 1976).

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review article

Present status of γ-ray astronomy

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Diffuse extragalactic γ-ray flux may be due to individual objects, and two possible sources are 3C273 and NGC4151. The Milky Way is an intense line source exhibiting a large contrast in emissivity between the directions towards and away from the galactic centre, respectively. γ-ray point sources and cosmic rays interacting with interstellar material are both responsible for this emission—the latter decreasing away from the centre. Twenty-one point sources have been discovered to date, including four pulsars which all have two maxima with ~0.4 phase separation. If the number and position of γ-ray point sources are proportional to the stellar birth rate, the bulk of the galactic emission can then be explained by 5,000 sources emitting the 10^{42} *photons* $>100\,\mathrm{MeV\,s^{-1}}$ *produced in the galaxy.*

THE new discoveries by ESA's satellite Cos B (refs 1–3) and successful[4] balloon observations provide new material for a discussion of the astrophysical processes leading to γ-ray production. The sky in the light of γ rays shows two distinct features, a general and rather diffuse flux from extragalactic space against which the narrow and brilliantly luminous band of the Milky Way stands out.

γ Rays from extragalactic space

The cosmos is filled with photons of all wavelengths. If one considers an average point in intergalactic space, the energy density of photons in the various energy bands is like that shown in the hatched area[5] of Fig. 1; the full line represents the energy density in photons from the Galaxy in the solar neighbourhood. Remarkably the energy density in metagalactic space remains at the high level of several $10^{-5}\,\mathrm{eV\,cm^{-3}}$ in the X- and γ-ray domain. This is because hard X rays and γ rays are practically not absorbed[6] (the redshift, at which the cosmos becomes opaque to γ rays is $z \sim 100$) and distant sources therefore make a similar contribution to the diffuse flux as close ones. Thus, extragalactic γ-ray astronomy must be considered with cosmological questions.

Observationally, there seems to be agreement about the shape of the energy spectrum of the diffuse radiation[7–9]. Figure 2 shows a shoulder at around 3 MeV which steeply drops beyond 10 MeV. The data at lower energies accord well with the results at high (>50 MeV) energies, if allowance is made at high energies for a flat galactic contribution to the extragalactic flux.

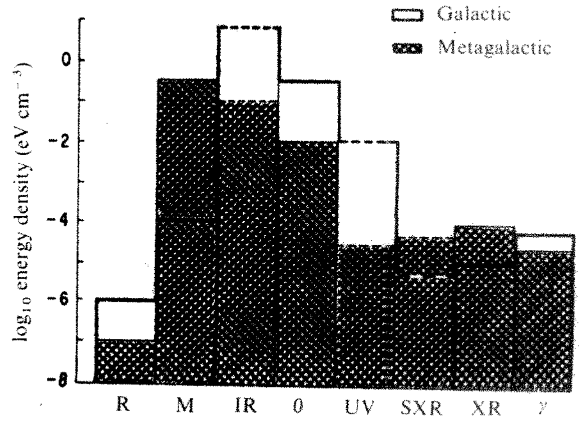
The origin of the diffuse radiation in general and the shoulder at 3 MeV in particular is still unclear; is it due to a truly diffuse mechanism, or to an as yet unresolved superposition of many individual sources? We encounter the same problem when we consider the origin of galactic γ rays.

This uncertainty has been due to, on the one hand, the little observational evidence available on the γ-ray luminosity of galaxies, quasars and other objects, and on the other hand, the models for truly diffuse production either in intergalactic space now or during the big bang have free parameters. It has thus been possible to reproduce the same observational results with several different models. For example, the diffuse radiation could originate from electrons that escape from galaxies into intergalactic space, or at least the shoulder could be caused by redshifted photons from π^0-decay that in turn have been produced either by a copious supply of cosmic rays in the big bang, or by simultaneous baryon–antibaryon annihilation[5,10].

Some new light has been shed on this situation by the observations of two extragalactic γ-ray sources[3,4] which have been identified to be 3C273 and the Seyfert galaxy NGC4151. If these identifications are correct, 3C273 would emit $2\times10^{46}\,\mathrm{erg\,s^{-1}}$ in 50–500 MeV γ rays, and the spectrum of NGC4151 would look like that shown in Fig. 3. Certain assumptions about space density and luminosity, the diffuse γ ray background could then be explained by a superimposition of individual sources[4].

These new discoveries have directed attention towards extragalactic γ ray astronomy for other reasons. One possible mechanism for producing the sharp cut-off in the spectrum of NGC4151 at 3 MeV is the Compton process[11]. Due to large energy losses, a sharp cut-off will have been generated in the energy spectrum of the electrons required to scatter photons into the γ-ray domain which then reproduces itself in the spectrum of NGC4151. If this were true, the position and shape

Fig. 1 Energy density of the cosmic photons. The hatched area shows the energy density of photons in the various energy bands (radio, R; microwave, M; infrared, IR; optical, O; ultraviolet, UV; soft X ray, SXR; X ray, XR; and γ ray band) in metagalactic space; the full line shows the galactic contribution evaluated in the solar neighbourhood. After Silk[5].



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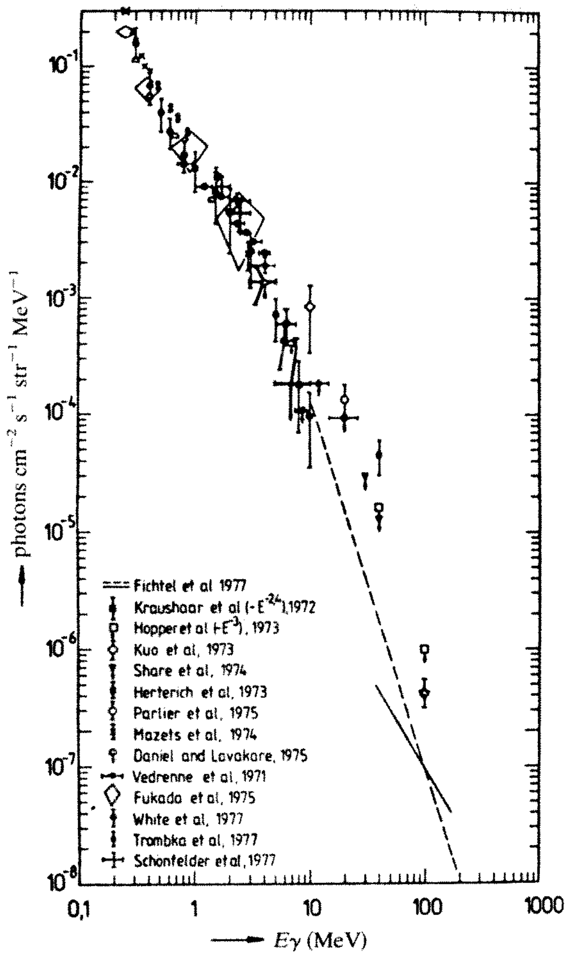

Fig. 2 Energy spectrum of the diffuse γ radiation after Schönfelder *et al.*[7], Fichtel *et al.*[8] Taken from ref. 9. The solid line is the contribution of galactic γ rays to the total diffuse flux from high galactic latitudes, the dashed line is the remaining extragalactic flux if the galactic contribution is subtracted from the total diffuse spectrum.

of the cut-off must be highly time-variable[12] and it would give some indication of the energy requirement and rate of occurrence of events that power Seyfert galaxies like NGC4151.

γ Rays from the Galaxy

The galaxy appears as a narrow line source in high-energy γ rays, its width at half maximum[13] is $+3° \geq b^{\mathrm{II}} \geq -2.5°$ at $l^{\mathrm{II}} = 0°$, and $+7° \geq b^{\mathrm{II}} \geq -3°$ at $l^{\mathrm{II}} = 120°$. (The significance of this deviation from the galactic plane would be interesting to consider.)

Quantitatively, the line source strength of the galaxy is shown[13] in Fig. 4. This is an old picture: although the galactic survey has been completed by Cos B, but the new data have not been published yet. However, in the complete data set, regions of intense γ-ray emissivity in the plane seem to occur when the line of sight tangentially intersects spiral arms which in turn have been localised by high excitation parameter H II regions and intensity maxima in the radio continuum and neutral hydrogen[16].

γ-ray point sources are also embedded in the galactic plane. Their positions (and identifications, if any) are indicated by arrows in Fig. 4. A list of point sources is given in Table 1 (refs 17, 18). The nature of the galactic γ-ray emission has been widely debated. γ rays originate from the interactions of cosmic-ray nucleons and electrons with interstellar matter, yet the observed line source strength shown in Fig. 4 in the direction towards the centre differs strongly from the fluxes expected in a simple model where cosmic rays (with the average galactic intensity as observed near the Solar System) interact with interstellar atomic hydrogen. These expected fluxes would not vary much as a function of longitude, because the projected density of atomic hydrogen along the line of sight also is rather constant. In this simple model, the γ-ray line intensity over the entire longitude range would be that indicated by the dashed lines in Fig. 4. Clearly, many more γ rays come from the longitude interval between ±60° than can be explained by this simple model[19].

One way of explaining this discrepancy would be to assume that more material and cosmic rays are available in the inner parts of the Galaxy. On the other hand, γ rays also originate in point sources. Can they account for the difference? The discussion is complicated by the fact that the angular resolution of a γ-ray telescope (several degrees) is insufficient to directly answer these questions.

Discrete sources

The truly diffuse component is that part of the galactic radiation that remains if point sources are subtracted. It is therefore necessary to consider these first.

γ-ray point sources are of high astrophysical interest in their own right and indeed the impact of γ-ray astronomy on the whole of astrophysics may eventually be larger here than in the study of the regions of residence of cosmic rays in the Galaxy.

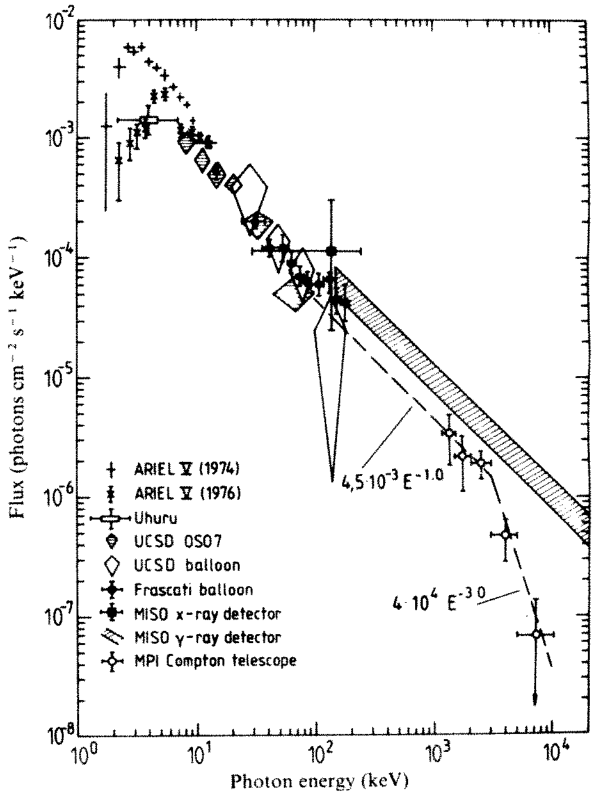

Fig. 3 The energy spectrum of γ rays from the direction of NGC4151. After Schönfelder[4]. The MISO γ-ray detector actually measured an integral flux > 150 keV and not a spectrum. The hatched area represents the spectrum derived by these authors, and compatible with their integral flux under the assumption of an $E^{-1.0}$ power law extending to infinity. It thus does not conflict with the measurements of the MPI Compton telescope.

Due to the limited angular resolution, point source identifications in the Galaxy have been possible only for pulsars[20,21], the number of which has now been increased due to Cos B to be four[22–24]: PSR1822 − 09, PSR0531 + 21, PSR0740 + 28 and PSR0833 − 45. It is very surprising that all four pulsars show double maxima with a phase separation of 0.4 (Fig. 5), although their energy spectra are different.

Furthermore, the luminosities of the four pulsars (using the dispersion measure as distance indicator) do not decrease in

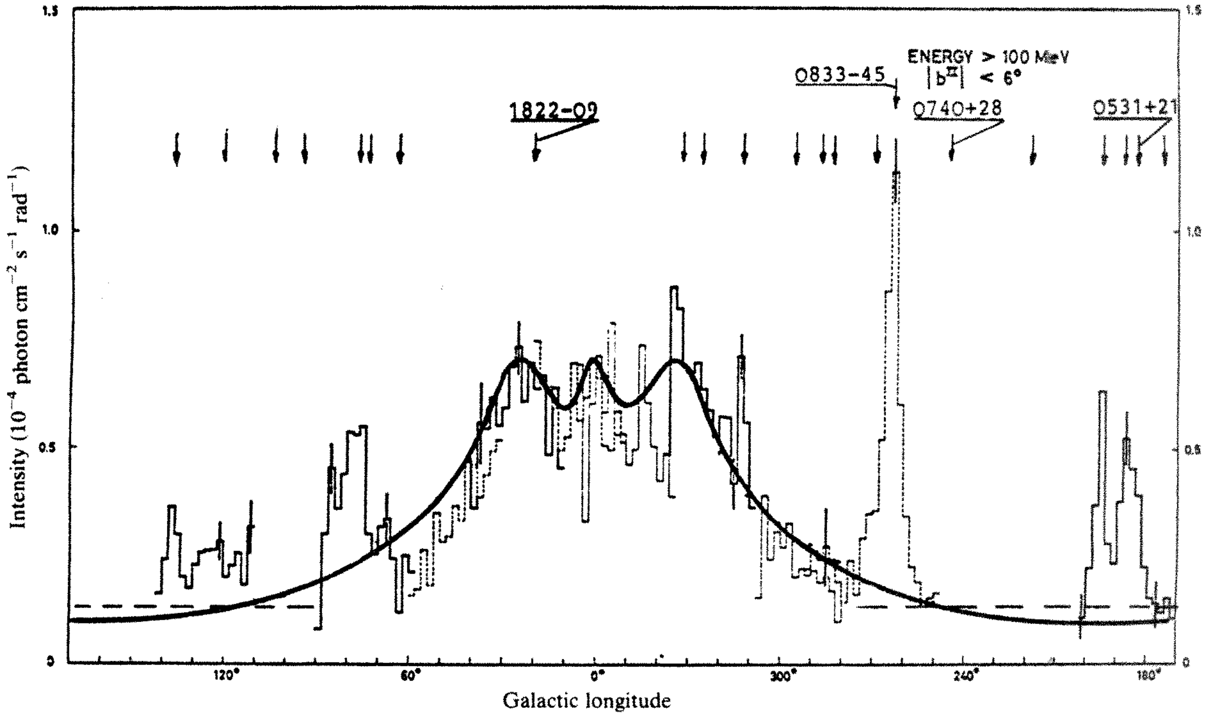

Fig. 4 Longitude profile of the intensity of γ rays of energy >100 MeV over the latitude range $|b^{II}| < 6°$. The dashed line roughly indicates the line intensity expected from cosmic rays interacting with interstellar gas in the region $l^{II} = 180° \pm 80°$ after Fichtel *et al.*[14], allowing for the narrower latitude range $|b^{II}| < 6°$. The solid line is the distribution in apparent brightness of O-stars as given by their Lyman-continuum flux after Mezger[15]; it has been normalised to the average line intensity of $\sim 10^{-5}$ photons >100 MeV cm^{-2} s^{-1} rad^{-1} contributed by the Cos B point sources from the two viewing periods centred on 126° and 185°, respectively.

proportion to decreasing $\dot{P}/P^3$ which in turn is proportional to $\dot{E}$, the rate of loss of rotational energy (assuming the same momentum of inertia in all four pulsars). Thus, at least in the four cases observed (which may be subject to selection effects), the efficiency of converting $\dot{E}$ into γ-ray luminosity increases with decreasing $\dot{E}$ until some as yet unknown limit is reached[23–25].

Finally, at least in the case of the Crab (0531+21) and Vela (0833−45), the emission from these sources is compatible with it being entirely pulsed. The 2σ lower limit of the pulsed fraction is 78% (>50 MeV, Crab) and 91% (>50 MeV, Vela), respectively[22].

Attempts have been made to identify at least some of the Cos B sources with other objects[17] like X-ray sources[26,27], radio-optical sources, supernova remnants[28], H II-regions[29], and dark clouds[30] (which radiate γ rays if they are penetrated by cosmic rays). Due to the large positional error in the γ ray observations, these have remained possibilities.

General galactic emission

In the outer parts of the Galaxy, γ rays are clearly produced by both, cosmic ray interactions with interstellar matter, and point sources. A cosmic-ray gradient away from the galactic centre must exist[43], because the γ-ray intensity seen from the outer parts is less than that expected if cosmic rays were to fill the entire galactic disk with the same intensity as that near the Solar System.

Whether or not the high flux value of γ rays from the inner parts of the galaxy is due to point sources or a truly diffuse process requiring more interstellar material and/or more cosmic rays there, is as old a question as the first positive results of γ-ray astronomy[19,31].

It has been shown[19] that the radio emissivity, the distribution of neutral and molecular[32,33] hydrogen alone do not sufficiently well represent the spatial distribution of general γ-ray emissivity in the Galaxy. Consequently, one has to construct the population of γ ray emissivity theoretically by assuming a correlated variation of cosmic-ray energy with matter density, be it in the spiral arms[14], or in a ring of high matter density as provided by molecular and atomic hydrogen[33].

These models of the galactic γ-ray emissivity have been constructed so that they can explain quite well the observational results. But they are not conclusive because the actual amount of interstellar material available as an interaction partner for cosmic rays, and the actual intensity of cosmic rays throughout the galactic plane are uncertain. Therefore there is still the possibility that the observed picture of our Galaxy in high energy rays could be dominated by point sources, γ-ray stars, rather than diffuse processes. Because individual γ ray sources cannot be resolved in an environment of large emissivity, and time variability may help identification in many but probably not all cases, many statistical arguments have been used with conflicting results. For example, Ögelman[34] attempted to explain the OSO 3 results by extrapolating the intensity of known X-ray sources into the γ-ray domain using a −2.0 power law index; the uncertainty of such a method was pointed out by Clark *et al.* [19]. Similarly, Coe *et al.*[26] extrapolated the apparent brightness of X-ray sources into the γ-ray domain, using the 4th Uhuru catalogue and Ariel 5 observations. They used their results to calculate an average for the galactic γ-ray emission by point sources, while Protheroe *et al.*[35] came to the conflicting conclusion that γ-ray point sources, based on a comparison with the 4th Uhuru catalogue, could account only for 19% of the galactic line emission.

The situation is also unclear if pulsars are used to estimate the possible contribution of point sources. For example, Kniffen *et al.*[36] estimated the pulsar contribution to galactic γ rays at 5%, while Higdon *et al.*[37] believe that a much larger contribution could be expected.

The problem with statistical arguments is that a known population is required, that has the same spatial distribution within the galaxy as that expected for the γ-ray sources. It has not been shown that the X-ray sources provide such a population. There are indications that the distribution of pulsars in the galactic plane[38] may be quite similar to the γ-ray emissivity.

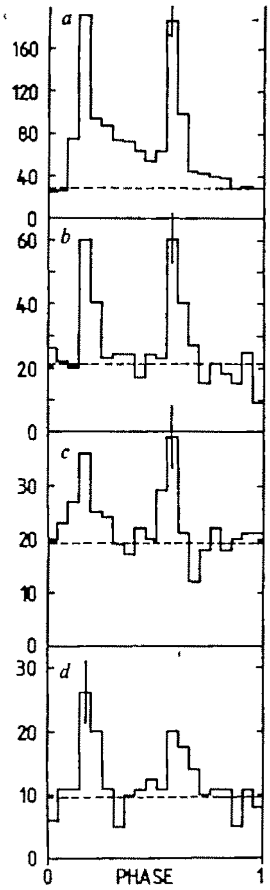

Fig. 5 Light curves of the four pulsars now observed by Cos B (refs 19–21): *a*, PSR0833−45; *b*, PSR0531+21, *c*, PSR1822−09, *d*, PSR0740−28

A statistical investigation of this question may start from the observations of point sources in the anticentre direction, assuming that all have been discovered in favourable conditions of observation, namely low background and low space density of sources which then allows detection in spite of the low power of angular resolution of the γ-ray telescope (several degrees). One then may extrapolate this result to cover the entire galactic plane, scaling it according to the variation of a known population of astronomical objects which are believed to have a similar space dependence as γ-ray sources. Attempts using X-ray sources or pulsars have led to conflicting results.

One may, however, investigate whether γ-ray point sources could be young objects and thus be correlated to the stellar birth rate. It had been noted (see, ref. 41) that the distributions in the galactic disk of H_2, H II-regions, γ rays and supernova remnants are all similar. However, the question of the stellar birth rate has recently been reviewed[39,40] and has quantitatively been estimated by counting the number of Lyman continuum photons emitted by O-stars per element of volume of the galactic disk. From observations of giant radio H II-regions[39] and the extended low density H II-regions, Mezger[15] was able to derive the emissivity of the galactic disk in Lyman continuum photons as a function of galactocentric distance.

It is proposed here that the spatial distribution of γ-ray localised sources closely resembles that of the O-stars, that is that of the Lyman continuum photons (Fig. 6). The width of that layer in the galactic plane is very narrow, ±100 pc, in agreement with the narrow width of the galactic γ-ray emissivity.

One may now construct the line source intensity of apparent brightness of the Lyman continuum photons by using the data of Fig. 6 and integrating along the line of sight through the galactic plane, taking account of the limited thickness of the disk: this is the full line of Fig. 4 and it supports the conjecture that the population of O-stars has the same spatial distribution as the γ-ray emissivity.

Figure 4 shows a rough comparison which is circumstantial in the sense that Mezger[15] unravelled observational results to arrive at the galactic production rate of Lyman continuum photons which then had to be reconverted to the distribution of apparent brightness along the line of sight (Fig. 4). However, a detailed comparison of extended low-density H II- and radio H II-regions with the γ-ray sky map (which will be forthcoming shortly from the Cos B observations) may reveal a much greater agreement in detail. Already, correlations between Cos B point sources and H II-regions have been noted[29,32], and the spiral structure, which also is visible in the γ-ray sky surveys, is in turn delineated by H II-regions[16]. Also, 74% of the Lyman continuum emissivity is estimated to come from the main spiral arms, yet only 13% from the interarm region, and 13% from the galactic centre[39]. Thus, if more γ rays are found to come from spiral arms[14], it may be because the γ-ray point sources have a larger density there.

Approximately, then, 3×10^{11} Lyman continuum photons are emitted per single γ ray with energy exceeding 100 MeV, corresponding to a galactic luminosity of 10^{42} (γ's > 100 MeV s^{-1}). In other words roughly 10^{49} γ rays above 100 MeV are produced per birth of one solar mass.

These considerations may now be applied to an investigation of the relative importance of point sources and diffuse emission.

Table 1 γ-ray sources detected by Cos B

Galactic coordinates l^{II}	b^{II}	No. of observations	Spectral class	Remarks	Relative intensity
21.0	+1.0	1	Soft	PSR1822-09	~0.8
65.5	−0.0	3	Hard		$0.30^{+0.10}_{-0.05}$
75.0	+0.0	1	X		$0.45^{+0.15}_{-0.10}$
78.5	+1.5	1	X		$1.00^{+0.30}_{-0.20}$
95.5	+4.5	2	Hard		—
106.0	+1.5	1	X		—
121.0	+3.5	1	Hard		$0.20^{+0.10}_{-0.05}$
135.5	+1.5	2	Medium		$0.30^{+0.15}_{-0.10}$
176.0	−7.0	1	Soft		0.50±0.10
184.5	−5.5	2	Medium	PSR0531+21	1.00
189.0	+1.0	1	X		0.40±0.10
195.5	+4.5	2	Hard		0.90±0.20
219.0	−0.5	1	Medium		—
243.0	−2.5	1	Hard	PSR0740+28	~0.4
263.5	−2.5	5	Hard	PSR0833−45	3.30±0.50
270.0	−1.0	2	Soft		—
284.0	−1.0	1	Hard		—
288.5	−0.5	1	Medium		—
291.0	+65.0	1	Medium	3C273	~0.25
295.5	+0.5	1	Medium		—
312.0	−1.5	1	X		0.45±0.15
327.5	−0.5	1	X		0.35±0.15
333.5	+0.0	1	X		0.70±0.20
353.0	+16.0	1	Hard	in Ophiuchus dark cloud complex	

The information is preliminary; analysis continues. Error circles for positions have radii between 0.5° and 2.0°. With the exception of CG263-2 source intensities (>100 MeV) are within the range 0.2 to 1.0 times that of CG185-5. Spectral clarification is based on the relative numbers of photons detected in the ranges 50 to 150 MeV and >150 MeV. The pulsar identifications are confirmed by timing phase analysis. Sources marked X are located in complex regions where more detailed analysis is needed to provide information additional to that given in the first Cos B catalogue.

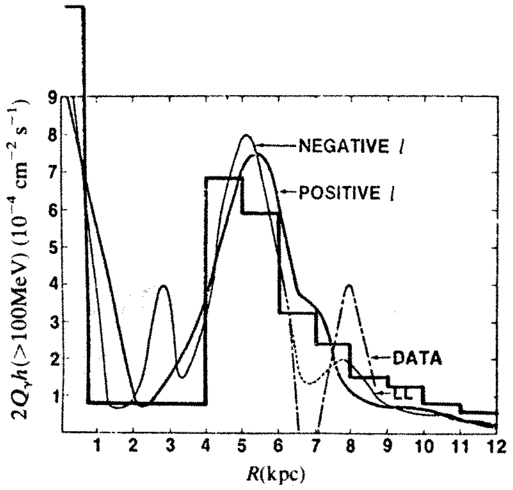

Fig. 6 Modified from Stecker[32], the smooth lines being the result of an unfolding of γ-ray line intensity data assuming cylindrical symmetry of γ-ray emissivity in each half of the galaxy. (Compare also Strong *et al.*[42]). The histogram represents the source function of Lyman continuum photons after Mezger[15], if 3×10^{11} Lyman continuum photons are produced per γ ray >100 MeV.

Figure 4 shows that the Lyman continuum population has nearly the same apparent brightness for the longitude interval $l^{\text{II}} = 180° \pm 80°$. Two Cos B observation periods[17] centred at $l^{\text{II}} = 126°$ and 185° fall within that longitude. During each viewing period, an angular interval of ~0.6 rad in longitude was observed.

There are six point sources with, five without the Crab in both angular intervals (including one source at $b^{\text{II}} = 7°$), leading to a contribution of point sources of 1.4×10^{-5} photons >100 MeV $\text{cm}^{-2}\,\text{s}^{-1}$ with, and 10^{-5} photons >100 MeV $\text{cm}^{-2}\text{s}^{-1}$ without the Crab. In both regions, an angular width of ~1.2 rad was scanned, thus the line source contribution of point sources is $\sim10^{-5}$ photons >100 MeV $\text{cm}^{-2}\,\text{s}^{-1}\,\text{rad}^{-1}$.

One may now scale the number of γ-ray point sources and their apparent brightness according to the stellar birth rate, that is the apparent brightness of O-stars as given by the solid line of Fig. 4. This line has been normalised to the value of $\sim10^{-5}$ photons >100 MeV $\text{cm}^{-2}\,\text{s}^{-1}\,\text{rad}^{-1}$ at the two viewing directions of Cos B used above. Figure 4 shows that the flux of these point sources can fully explain the shape and enhancement of the γ-ray line source towards the galactic centre.

On the other hand, one may argue that it is the cosmic-ray density that scales like the stellar birth rate, and not the number of γ-ray point sources. In the regions of normalisation used above, the contribution of cosmic ray interactions with interstellar matter is of the order of 1.3×10^{-5} photons >100 MeV $\text{cm}^{-2}\,\text{s}^{-1}\,\text{rad}^{-1}$ (ref. 14) if one takes account of the latitude range $|b^{\text{II}}| < 6°$. The average contribution of point sources to the combined flux of $\sim2.3\times10^{-5}$ is then 43%. Had the distribution in apparent brightness for O-stars been fitted to the value for cosmic ray produced γ rays alone, a value of 9×10^{-5} photons $\text{cm}^{-2}\,\text{s}^{-1}\,\text{rad}^{-1}$ would have been predicted for the galactic centre region (somewhat high (Fig. 4)), and 1.6×10^{-4} photons $\text{cm}^{-2}\,\text{s}^{-1}\,\text{rad}^{-1}$, if one had normalised to the total flux of 2.3×10^{-5} (much too high; see Fig. 4).

Thus one may scale only one of the two components, cosmic ray interactions or point sources, but not both. Therefore, one will have to argue either (as refs 14, 33, 35–37, for example) that the point source contribution remains at a comparatively low level throughout the galaxy, and the γ-ray emissivity through cosmic-ray matter interactions scales like the stellar birth rate, or one may hypothesise that the γ-ray point sources are correlated with the stellar birth rate which then requires that the cosmic ray produced γ-ray emissivity remains at a low and perhaps even constant level throughout the galaxy. If one considers the consequences of the second hypothesis, that it is the point sources which follow the stellar birth rate, then 7–8 γ-ray point sources would have been expected to lie within the Cos B viewing direction centred at 74°, while only three have yet been discovered[17], and 14 should have been visible during the observation period centred at 322°, yet again only three have been seen[17]. 20 point sources would be expected from an observation period directed towards the galactic centre. As the longitude interval of any Cos B observation period is only ~30° wide one can clearly understand, why the γ-ray instrument cannot resolve these sources. Also, the large number of sources that are expected to lie within any viewing direction may account for the fact that the energy spectra as measured by Cos B do not change significantly as a function of galactic longitude[2].

Hermsen *et al.*[17] argued that the individual γ-ray point source has a luminosity of $\gtrsim10^{35}$ erg s^{-1}, then approximately 5,000 point sources in the entire galactic disk are required to produce the bulk of the 10^{42} photons s^{-1} above 100 MeV that the galaxy emits.

The considerations made above, of course, just demonstrate a possibility, albeit an interesting one. They are, first limited by statistics as there is a 40% error in six point sources. Second, it is a hypothesis that γ-ray sources are correlated to the stellar birth rate and thus to the population of O-stars, and that in addition such a correlation can be extrapolated from the outer regions $l^{\text{II}} = 180° \pm 80°$ to the entire galactic disk.

On the other hand, the suppositions made here provide a powerful stimulus for further observational and theoretical investigations if such a correlation can be substantiated, and what the physical reasons behind it are.

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articles

Analysis of ^{36}Cl in environmental water samples using an electrostatic accelerator

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A new atom identification and detection technique for ultrasensitive mass spectrometry has been applied to the radionuclide ^{36}Cl. Measurements of the ^{36}Cl/Cl ratio in AgCl precipitated from six different natural water samples fell in the range 1.2×10^{-13}–2×10^{-12} with a background level below 3×10^{-15}. For each sample 70 mg of AgCl was used, requiring 1–5 l of water.

CHLORINE-36, the longest lived radioactive isotope of chlorine, is continuously being produced in the atmosphere. In contrast with atmospheric ^{14}C, which forms a large reservoir as gaseous CO_2, ^{36}Cl leaves the atmosphere in rain and enters the hydrosphere directly. Due to the low rate of exchange with solids, the soluble chlorides containing ^{36}Cl form an effective tracer in groundwater hydrology, and it has been suggested that the 308,000 yr half life would make it a promising clock for a chronology that could cover the entire Pleistocene era[1]. Recently, the advantages of using ^{36}Cl to study groundwater were stressed[2] in connection with establishing the suitability of underground sites for the storage of nuclear wastes. However, before a ^{36}Cl chronology can be established, the origin and production rates of ^{36}Cl must be determined accurately and the measured concentrations related to hydrogeological and palaeoclimatological data. Such detailed and difficult studies should be stimulated by a demonstration here that ^{36}Cl can be detected in small samples with sufficient sensitivity.

The few measurements of ^{36}Cl in environmental samples published to date have been made by counting β emissions from the radioactive decay. Such experiments are difficult because of the low counting rates resulting from the long half life of ^{36}Cl. In addition, it is difficult to distinguish the β rays of ^{36}Cl from those of trace quantities of other radionuclei in the sample because of their low energy[3]. Tens of grammes of chloride taken from thousands of litres of water are required to give counting rates distinguishable above the background and even then counting times as long as a week are required. This article describes the first successful application of the Rochester MP tandem van de Graaff accelerator system as an ultrasensitive mass spectrometer[4–6] to the problem of determining the ^{36}Cl/Cl ratio in tens of milligramme chloride samples taken from 1 to 5 l of water from various sources. For the 45 mg background sample of zone refined AgCl reagent a concentration of $<3\times10^{-15}$ was determined, equivalent to an activity of about 1 β ray yr^{-1}.

To use ^{36}Cl for dating groundwater, the input of ^{36}Cl must be determined for a region and assumed to be relatively constant in the past. Atmospheric ^{36}Cl originates from interactions of cosmic rays and their secondaries with ^{40}Ar and ^{35}Cl, from nuclear explosions, and possibly from the influx of meteoric dust. At the surface of the Earth ^{36}Cl is produced through the activation of ^{35}Cl by cosmic ray secondary neutrons and in the subsurface by neutrons from the natural radioactivity in uranium and thorium deposits[7]. To use the ^{36}Cl/Cl ratio, the dilution from old chlorine must also be understood. Atmospheric chlorine arises primarily from ocean spray, while additional chlorides enter groundwater from dissolution of salt deposits. Measuring the ^{36}Cl/H_2O ratio instead of the ^{36}Cl/Cl ratio corrects for an unknown dilution by old chlorine, but uncertain precipitation and evaporation rates complicate the analysis. Although some progress has been made in predicting quantitatively the above origins of ^{36}Cl and Cl in water, it is beyond the scope of this paper to discuss them in detail.

The samples studied, listed in Table 1, were chosen partly to cover a wide range in expected ^{36}Cl content and partly for reasons of convenience. the Rillito River sample, taken from a well near an ephemeral stream, is largely runoff from recent rainwater and is therefore expected to contain a high ^{36}Cl content from nuclear explosion tests. In an attempt to exclude the bomb input source, a sample was obtained from a deep well near Tucson, Arizona that had been estimated[8] by means of tritium and ^{14}C analyses to be more than 2,000 yr old. As dilution from old salt deposits was also expected to be low, the ^{36}Cl/Cl ratio in this sample should provide a direct indication of the cosmic ray production rate in the atmosphere—the most important result required for establishing a ^{36}Cl chronology. Also studied were two samples from the Lake Ontario water intake of Eastman Kodak, a reagent grade sample that presumably originated in a mine (and thus should contain little ^{36}Cl), and two samples artificially enriched in ^{36}Cl.

Separation and identification of interfering isotopes

The methods and results for atom identification and detection of ^{14}C in natural samples were discussed in detail at a recent conference on radiocarbon dating[9], where the first detection of

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^{36}Cl, using a tandem accelerator[10], and an attempt to detect ^{36}Cl using a cyclotron[11] were also reported. The present apparatus (Fig. 1) incorporates some important improvements over that used for the ^{14}C measurements[13] which were found to be necessary to resolve the more closely spaced isotopes at the higher mass. An advantage of accelerating ^{36}Cl to energies above 1 MeV AMU^{-1} is that the atomic number Z can be identified by measuring the rate of energy loss of the ion. This Z determination, combined with the usual selection of a specific mass-to-charge ratio M/q with a combination of magnetic and electrostatic analysers, allows complete and unambiguous isotope identification without the severe loss in sensitivity typical of conventional high-resolution mass spectrometers. Although this analysis is adequate for positive identification of ^{36}Cl, in practice, the counting rate in the detector arising from trace impurities in the sample was high enough to produce gain shifts in the detector and produce tails that overlap the ^{36}Cl peak.

The basic limitation in measuring small concentrations of ^{36}Cl with mass spectrometers is due to the necessity of separating ^{36}Cl from the isobars ^{36}Ar and ^{36}S and from mass 36 molecules. Although chlorine readily forms stable negative ions, which is a requirement for acceleration in a tandem van de Graaff, argon (a noble gas) does not. No evidence was seen for argon in the detector (<1 argon atom per 10^{14} chlorine atoms) indicating that either Ar$^-$ is not produced in the caesium sputter source or that it does not have a lifetime sufficiently long to reach the tandem terminal as a negative ion. Sulphur, however, forms negative ions almost as readily as chlorine, so, with a ^{36}S abundance of only 0.014%, a one p.p.m. sulphur impurity produces an interference at the 10^{-10} level. The great sensitivity of the present technique is illustrated by the fact that a 1 p.p.m. concentration, considered very low for a sulphur impurity, would still result in a ^{36}S count rate of 300 c.p.s. for typical conditions in this experiment. The range separation technique[14] is not applicable because sulphur has the longer range so that special sample preparation techniques[15] were necessary to reduce the sulphur content to <10 p.p.m. In addition, the heavy-ion counter[12] was modified to give a ^{36}S rejection rate of better than 10^6 to 1 (see Fig. 2).

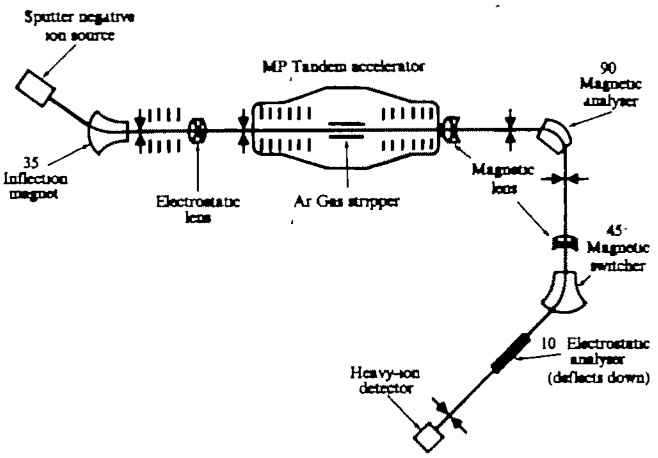

Fig. 1 Diagram of the apparatus used for ^{36}Cl measurements. From 5 to 13 μA of Cl$^-$ ions were produced from AgCl or NaCl samples in a caesium sputter source (model 834 HICONEX, General Ionex Corp.) the mass-36 negative ions were analysed by a magnetic inflection system ($M/\Delta M = 25$), accelerated to 170 KeV, then injected into the Rochester Mp tandem accelerator. At the terminal, which was held at 10.00 MV by a generating voltmeter stabilisation system. the ions were stripped of several electrons by argon gas, then accelerated again to ground potential. Charge state 7, mass 36 ions with $E = 80.17$ MeV were selected by a 90° magnet ($P/\Delta P = 600$), bent by a 45° magnet (which served to eliminate scattered particles), deflected 10° out of the horizontal plane by electrostatic plates ($E/\Delta E = 200$), and then analysed by a heavy-ion ionisation counter[12]. By increasing the inflection magnet and analysing magnet fields the ^{37}Cl beam could be switched onto the Faraday cup following the analysing magnet—the beam current measured here was used for normalisation.

Although interference from mass-36 molecules was eliminated by choosing a high charge state from the post-acceleration analysis system, atomic fragments that result from breakup of the mass-36 molecules at the terminal are not separable from ^{36}Cl in the magnetic and electrostatic analysers if they happen to have the same value of M/q after acceleration as the ^{36}Cl ions. For example, the $^{12}C_3^-$ molecular ion, known to be produced in nA quantities[18] by sputter sources, will behave exactly as ^{36}Cl^{6+} in electric and magnetic fields when stripped to ^{12}C^{2+} in the terminal. Although these carbon ions could be distinguished by their energy loss in the detector, the counting rate would be excessive even for a very low level of carbon impurity. This type of background can be eliminated by choosing a charge state that has no common factor with 36, that is 5, 7, 11, 13 or 17. For 10 MeV chlorine in argon gas, the charge-state distribution peaks near 6 with a full width at half-maximum of about 3 charge units[18], thus leaving 5 and 7 as the only good choices. Charge-state 7 was chosen for its higher energy (80 MeV as opposed to 60 MeV for charge-state 5) allowing better separation from ^{36}S in the detector.

Other potential sources of interference are ^{35,37}Cl ions which are injected into the tandem from tails of the intense ^{35,37}Cl$^-$ beams at the ion source because of the limited resolution of the inflection magnet (^{35}Cl can also be injected directly as H^{35}Cl$^-$). Although most of these ions will leave the tandem at discrete energies that are not selected by the 90° analysing magnet, some will charge exchange a second time in the residual gas of the

Table 1 Results for ^{36}Cl measurements

Sample	Total run time (min)	Total ^{36}Cl counts	Number of runs	Concentration ^{36}Cl/Cl($\times 10^{15}$)	Activity (d.p.m. − kg Cl)	Cl/H$_2$O (mg^{-1} 1)	^{36}Cl/H$_2$O ($\times 10^6$) (atoms^{-1} 1)
Groundwater, Rillito River (ephemeral), AZ	90	7,681	7	1,950±150	145	48	1,600
Surface water, San Pedro River, Tombstone, AZ	204	8,916	13	650±110	48	4–12	~90
Groundwater, flowing well in St David, AZ	68	2,403	7	400±40	30	8	53
Subsurface water, Lake Ontario, NY (May, 1978)	41	2,062	4	210±20	15	27	95
Subsurface water, Lake Ontario, NY (June, 1978)	172	4,555	9	120±20	9	27	54
AgCl reagent, zone refined	57	32	3	3±1	0.2	—	—
NaCl enriched to ^{36}Cl/Cl ∼ 33,000 × 10^{-15}	13	21,799	2	18,500±900	1,320	—	—
NaCl above diluted to ^{36}Cl/Cl ∼ 330 × 10^{-15}	58	975	4	250±30	18	—	—

Although a few hundred mg of AgCl was obtained from the natural water samples, only 16–66 mg was used in the ion source, and most of this remained after the experiment. Errors quoted are one standard deviation for several measurements. Specific activity is that expected based on the ^{36}Cl concentration measured. The approximate total chlorine concentration in the original water sample is given in the next to last column, followed by the ^{36}Cl concentration.

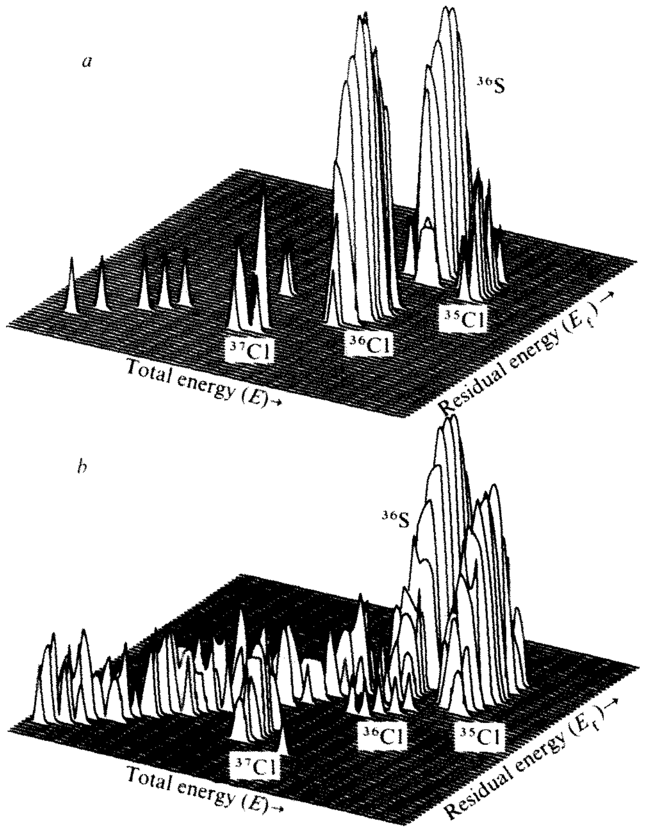

Fig. 2 Two-parameter logarithmic spectra for single runs on: *a*, a sample from Lake Ontario; and *b*, zone refined reagent grade AgCl. To help remove the effect of a high-energy tail arising from the Landau-Symon effect[16] on the intense ^{36}S peak in the energy loss spectrum (not shown), the heavy-ion counter[12] was modified to include four energy loss (ΔE) plates and a smaller residual energy (E_f) plate. In the computer, a digital window was placed on each of the first three ΔE signals at the expected location of ^{36}Cl. This reduced the effect of the tails since the high energy-loss events that produce them are not correlated among the different ΔE measurements. The events passing the three ΔE gates and an additional hardware E_f gate were plotted on the two parameter display of E_f against E shown. The long ^{36}S tail results from lower energy particles scattered into the counter. The zero has been offset on each axis.

acceleration tubes to produce $^{35,37}Cl$ ions with the same momentum and charge state as the ^{36}Cl ions. With only the ME/q^2 selection of magnetic analysers (all that is normally used with accelerators) the $^{35,37}Cl$ count rate was about 10^5 c.p.s. The intensity of this continuum was reduced more than 5 orders of magnitude with the E/q selection of an electrostatic analyser installed for these ^{36}Cl measurements (see Fig. 1). The remaining $^{35,37}Cl$ ions were easily resolved from ^{36}Cl in the detector.

Results and discussion

The results are given in Table 1. Normalisation for the $^{36}Cl/Cl$ ratio was obtained by reading $^{37}C^{7+}$ beam current before and after each ^{36}Cl measurement. As this current could not be monitored continuously, the accuracy of a measurement was limited by any fluctuations in the source output and accelerator transmission efficiency. The standard deviation of the $^{36}Cl/Cl$ ratios from several 10–20-min runs provided a measure of this uncertainty and was usually 10–15%, which is 2–4 times larger than the statistical error for single runs. In subsequent ^{14}C experiments the cycle period was decreased to 3 min, and this uncertainty, when calculated for the mean of several cycles, was reduced to about 3%. There is no reason that this level of uncertainty could not now be obtained for ^{36}Cl, where 3% corresponds to an age uncertainty of 13,000 yr.

The experimental method was checked by using two samples enriched in ^{36}Cl: the first was made by exposing reagent grade NaCl (BDH, lot no. 81515-7203) to an approximately known neutron flux from the University of Toronto Slowpoke reactor, the second by dilution of the first. The measured ratio of ^{36}Cl content, 74 ± 10, is in marginal agreement with the expected value of 100. This discrepancy would be resolved if the original reagent contained ^{36}Cl with a $^{36}Cl/Cl$ ratio as low as 30×10^{-15} (the ^{36}Cl content of the reagent used was not measured and was assumed to be negligible when determining the dilution factor of 100). A comparison between the absolute $^{36}Cl/Cl$ ratios is not useful because of the not-so-well known neutron flux. Clearly, more calibration checks need to be made before detailed hydrology studies are undertaken.

The low result for the zone-refined reagent-grade AgCl (Fisher catalog no. S174, lot no. 742272), demonstrating a sensitivity of three parts in 10^{15}, is probably the most significant result of this work. The sensitivity may be greater than this value as it could not be determined what fraction of the 32 counts collected was attributable to the ^{36}S tail (see Fig. 2) or how much was due to cross-contamination from other samples in the ion source. Even when considered as an upper limit to the sensitivity, the result shows that this technique is adequate for dating samples with ages up to 6 half lives or 2×10^6 yr. Memory effects and cross-contamination were shown to be small because the non-diluted enriched sample was run for ~ 4 h before experiments on the refined sample and so a dynamic range of almost 10^4 was realised.

Interpretation of the results for the neutral samples is difficult due to the present lack of detailed hydrogeological data, but H. Bentley helped us draw the following preliminary conclusions. Although the Rillito River sample was taken from groundwater, it is expected to be quite young—the higher ^{36}Cl content probably reflects input from nuclear testing in the atmosphere. Irrigation of nearby fields is indicated as the source of the high chloride content of the sample, and as this additional chlorine is atmospheric in origin, it may contain appreciable ^{36}Cl. The San Pedro River sample, on the other hand, is from surface water that is fed by groundwater springs—the relatively low concentration of ^{36}Cl may indicate that the bulk of this water is pre-bomb in origin. In contrast, the St David and Tucson well samples are certainly pre-bomb in origin[8]—these results should indicate the base atmospheric cosmic ray production for water that has not aged long compared to the ^{36}Cl half life. The Lake Ontario samples probably give a low $^{36}Cl/Cl$ result because of dilution from the great quantities of de-icing salt[19] used near the Great Lakes in the last decade. The $^{36}Cl/H_2O$ ratio, not affected by the de-icing salt, is small compared to the Rillito river value because of the higher precipitation and lower evaporation rates in the Great Lakes region as compared to Arizona.

In conclusion, the method described can be used to measure the ^{36}Cl concentration in a 100-mg sample of AgCl with an uncertainty of $< 10\%$ in less than 1 h. This performance should be adequate for studies using ^{36}Cl as a tracer in groundwater hydrology. Among other geophysical dating applications likely to benefit from the new technique is the study of deep ice core samples in Greenland and Antarctica[20]. Also, important information about the cosmic ray containment time within the Galaxy can be inferred from the number of the long-lived radionuclides ^{10}Be, ^{26}Al, ^{36}Cl, and ^{129}I present in the cosmic ray flux[21]. The concentration of these isotopes in Moon rocks[22] or meteorites[23] might be indicative of that flux, and a depth profile may give information on the cosmic ray energies.

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Sm–Nd systematics of Lewisian gneisses: implications for the origin of granulites

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Sm–Nd isotope systematics of Lewisian gneisses from North-west Scotland suggest that their igneous calc-alkaline precursors separated from previously undifferentiated mantle 2,920±50 Myr ago. Approximately 200 Myr elapsed before this material was finally differentiated and stabilised as granulite and amphibolite facies crust.

DURING the past decade, measurements of Sr-, Nd- and Pb-isotope ratios have demonstrated that ancient crustal segments are, in general, stabilised shortly after the separation of their igneous precursors from the mantle. For example, the initial $^{87}Sr/^{86}Sr$ ratios of the gneisses comprising many Archaean crustal segments are found to be close to the estimated mantle $^{87}Sr/^{86}Sr$ ratio for the time of their stabilisation, an observation which precludes more than ~100–200 Myr (refs 1-5) residence in a high Rb–Sr crustal environment. These conclusions, which are generally accepted amongst isotope geochemists, sometimes seem contrary to the geological complexity of such regions, as deduced from field observations and from the substantial geochemical changes which the igneous precursors must have experienced before they became structurally and thermally stable continental crust. Notable amongst these latter changes is the marked depletion of heat-producing elements (K, U and Th)[5–11] and some other lithophile trace elements[12–16] in granulite facies rocks.

The time interval between the initial generation of sialic material from the mantle and the eventual stabilisation of continental crust, during which these latter geochemical changes occurred, can only be broadly constrained by Rb–Sr and U–Pb techniques as indicated above. However, the successful application of the ^{147}Sm–^{143}Nd system to the dating of Archaean rocks[17–19] indicates that a combination of Sm–Nd, U–Pb and Rb–Sr studies may produce the time resolution required. The considerable amount of Rb–Sr (refs 4, 20–24) and U–Pb (refs 4, 10, 11, 24–26) geochronological data already existing for the Archaean Lewisian gneisses of North-west Scotland makes this a favourable location for an attempt to resolve this time interval.

The Lewisian gneiss complex forms the western foreland to the Caledonian orogenic belt, and crops out on the mainland of North-west Scotland, on the Outer Hebrides and several of the islands of the Inner Hebrides, and as large deformed slices within the Caledonian belt itself. Detailed geological field studies beginning with Sutton and Watson[27], geochemical studies[15,30], and numerous geochronological investigations using K–Ar (refs 24, 28, 29), Rb–Sr (refs 4, 20–24) and U–Pb (refs 4, 10, 11, 24–26) techniques have served to subdivide the Lewisian into three main regions. The central region contains pyroxene gneisses which have crystallised at high temperatures and pressures[31–33], and which record structural and chronological evidence for the earliest (Scourian) granulite facies metamorphism ~2,700 Myr ago. The northern and southern regions are composed mainly of amphibolite facies gneisses whose relationships with the central region granulites have been considerably discussed, but are now widely regarded as having occupied an initially higher structural level in the crust than the granulites[30]. The amphibolite facies regions record evidence of two later periods of deformation, metamorphism and igneous activity at ~2,200 Myr (Inverian) and ~1,700 Myr (Laxfordian). These metamorphic events have locally affected the central region granulites causing retrogression to amphibolite facies mineral assemblages[30,34]. The samples used for this study came from the central region, near the village of Drumbeg, Assynt, and from the northern region, near Rhiconich, and have been described elsewhere[15,30,35].

Of the large amount of geochronological data which now exists for various components of the Lewisian complex, the more pertinent recent data of reasonable precision are summarised in Table 1. The three zircon suites analysed by Pidgeon *et al.*[25,26] yield U–Pb concordia–discordia intercept ages that are almost within error at 2,700 Myr. This age is interpreted as dating zircon growth during Scourian metamorphism. These data define lower intercept ages corresponding to well-defined geological events, namely the Laxfordian and the Caledonian. Zircon data from Lewisian gneiss in the northern region, near Durness[24] are enigmatic, giving an older concordia–discordia age of 2,810 ± 50 Myr and a lower intercept age of 1,040 ± 50 Myr.

The Pb–Pb and Rb–Sr whole rock ages for Lewisian gneisses in Table 1, though of poorer precision, agree with the Scourian ages obtained from zircon studies.

Sm–Nd systematics

The Sm and Nd contents and $^{143}Nd/^{144}Nd$ ratios (Table 2) have been measured on 11 samples of Lewisian orthogneiss including

Table 1 Lewisian geochronologic data

Age* (10^3 Myr)	Samples	Locality	Method	Ref.
2.63 ± 0.14	Whole-rock amphibolite facies gneisses	Southern Outer Hebrides	Rb–Sr	4
2.60 ± 0.12	Whole-rock amphibolite facies gneisses	Southern Outer Hebrides	Pb–Pb	4
2.86 ± 0.20	Whole-rock amphibolite and granulite facies gneisses	Outer Hebrides and N, S and central regions	Pb–Pb	10
2.68 ± 0.06	Whole-rock granulite facies gneisses	Scourie	Pb–Pb	11
2.81 ± 0.05	Zircon size fractions from quartzo-feldspathic gneiss	Rispond, Durness, northern region	Concordia–discordia intercept	24
2.73 ± 0.01	Zircon size fractions from quartzo-feldspathic gneiss	Harris	Concordia–discordia intercept	26
2.66 ± 0.02	Two zircon suites from granulite facies gneisses	Kylesku, central region	Identical concordia–discordia intercepts	25

* Errors are quoted at the 2σ level. Ages here and in the text conform to the following decay constants:

$$\lambda^{235}\text{U} = 9.85 \times 10^{-10}\ \text{yr}^{-1};\ \lambda^{238}\text{U} = 1.551 \times 10^{-10}\ \text{yr}^{-1};\ \lambda^{87}\text{Rb} = 1.42 \times 10^{-11}\ \text{yr}^{-1};\ \lambda^{147}\text{Sm} = 6.54 \times 10^{+12}\ \text{yr}^{-1}$$

both granulites and amphibolites. Previous major and trace element analyses of the Lewisian indicate an igneous origin from a plutonic suite with calc-alkaline affinities[14,30,35–37]. Analytical procedures for the chemical separation and subsequent mass-spectrometric analysis of Sm and Nd have been described elsewhere[17,38]. The Sm/Nd ratios are mostly less than the bulk earth value (0.31) (refs 17–19, 39, 40), corresponding to light rare-earth enriched patterns; however, two basic granulites are slightly light rare-earth depleted and have Sm/Nd ratios up to 0.372.

The data are plotted on a Sm–Nd evolution diagram in Fig. 1. The best fit line[41] has a slope corresponding to an age of $2{,}920 \pm 50\ (2\sigma)$ Myr ($\lambda^{147} = 6.54 \times 10^{-12}\ \text{yr}^{-1}$) and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of $0.508959 \pm 49\ (2\sigma)$. All the gneiss samples, irrespective of locality or metamorphic grade, conform closely to the best-fit line indicating that their igneous parent materials were derived contemporaneously and from source materials of similar history.

The Sm–Nd age obtained is significantly older than reliable ages obtained by whole-rock Rb–Sr and Pb–Pb and zircon U–Pb methods (see Table 1). The Pb–Pb and Rb–Sr data on whole rocks are consistent with a short time interval between formation of the igneous precursors and their chemical differentiation. The Sm–Nd age demonstrates that this is close to 200 Myr and the age is thus interpreted as the time of mantle–crust differentiation to produce the calc-alkaline precursors to the Lewisian. Specifically the time interval between the Pb–Pb and Sm–Nd whole-rock ages, $\Delta T_{\text{Nd-Pb}}$, is equal to 240 ± 90 Myr (uncertainties in the decay constants used should not significantly change this value).

Present day oceanic basalts exhibit a wide range of Nd compositions[38–40,42–45] with $^{143}\text{Nd}/^{144}\text{Nd}$ ratios between 0.51251 and 0.51329. This variation implies the existence of long lived mantle heterogeneities in Sm–Nd and an overall light rare-earth depletion (Sm/Nd > chondritic[46]) of oceanic upper mantle. The volume of mantle differentiated to produce the continental crust, the rate of crustal growth and the magnitude of the concomitant preferential light rare-earth (Sm/Nd < chondritic) enrichment into the crust are critical aspects of the history of present-day mantle heterogeneity. The precise timing of crust–mantle differentiation events and the evolution of radiogenic isotopes before such events would provide powerful constraints on solutions to these problems. We have shown that mantle–crust differentiation may be precisely dated by the Sm–Nd method. Furthermore, inferences concerning the previous Sm–Nd environment may be made from the corresponding initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios.

From Sm/Nd and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios determined on Archaean single whole-rocks of known age, DePaolo and Wasserburg[39,40] suggested that Archaean continental crust had grown by differentiation of mantle sources that had previously evolved with an approximately chondritic Sm/Nd ratio. On an age against initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio plot (Fig. 2) their data plot close to a model growth line defined by the chondritic Sm/Nd ratio of 0.31 (ref. 46) and a $^{143}\text{Nd}/^{144}\text{Nd}$ ratio (0.50682) (ref. 47) equal to that in the Angra dos Reis achondrite at 4,550 Myr ago. Their contention has been strongly supported by the Sm–Nd whole-rock isochron studies of Hamilton *et al.*[17–19] on Archaean igneous rock suites. The ages and initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios obtained by the latter also plot close to the chondritic growth

Table 2 Sm–Nd* data on Lewisian gneisses from the granulite facies zone (Drumbeg, Assynt) and the northern amphibolite facies zone (Rhiconich)

Rock type, locality (grid. ref.)	Sample	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd} \pm 2\sigma$
Basic gn.†, Drumbeg (NC128327)	7D	1.749	5.366	0.1960	0.512756 ± 24
Gt-basic gn., Drumbeg (NC107332)	8X	2.138	5.480	0.2346	0.513518 ± 30
Basic gn., Drumbeg (NC113340)	7N	2.289	8.064	0.1707	0.512195 ± 30
Tonalitic gn., Drumbeg (NC099325)	12F	2.146	11.92	0.1082	0.511006 ± 34
Tonalitic gn., Drumbeg (NC136312)	14F	10.33	83.81	0.0741	0.510376 ± 20
Tonalitic gn., Drumbeg (NC165319)	15M	2.554	17.54	0.0875	0.510634 ± 32
Tonalitic gn., Drumbeg (NC094328)	18T	2.650	15.46	0.1030	0.510962 ± 28
Tonalitic gn., Drumbeg (NC193336)	19X	3.062	23.75	0.0775	0.510501 ± 24
Granodioritic gn., Rhiconich (NC280510)	5218	7.694	41.05	0.1127	0.511143 ± 26
Granodioritic gn., Rhiconich (NC260530)	5326	5.776	39.87	0.0871	0.510638 ± 30
Tonalitic gn., Rhiconich (NC220540)	5422	5.631	33.88	0.0999	0.510902 ± 36

* All ratios atomic. † gn., gneiss.

line (Fig. 2), with Sm/Nd ratios computed for the time intervals from mantle–crust separations to 4,550 Myr being within error of 0.31. The time integrated Sm/Nd ratio for the igneous precursors to the Lewisian is 0.311 ± 4.

Several studies[48–50], including the present one, indicate that crustal metamorphism does not result in significant fractionation of Sm and Nd or perturbation of Sm–Nd isotope systematics. The major fractionation of Sm and Nd during Earth evolution is thus through the initial differentiation of the crust from the mantle. Using this fact and assuming differentiation of Archaean crustal segments from a chondritic Sm–Nd mantle environment, McCulloch and Wasserburg[50] computed model Sm–Nd ages from Sm/Nd and ^{143}Nd/^{144}Nd ratios measured on composite Canadian Shield samples. The model ages of 2,700–2,500 Myr agree with the available geological ages and give evidence of the derivation, within a short time interval, of a major crustal segment from essentially chondritic Sm–Nd reservoirs.

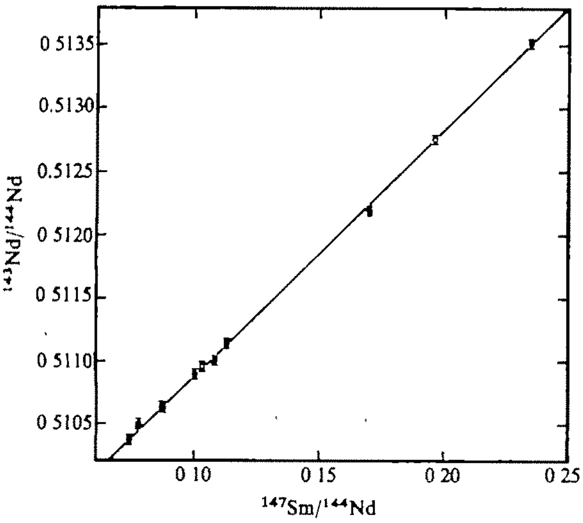

Fig. 1 Sm–Nd evolution diagram for the Lewisian gneisses. Age = $2,920 \pm 50$ Myr, initial ^{143}Nd/^{144}Nd ratio = 0.508959 ± 49.

Differentiation of continental crust and granulite formation

Whole-rock Pb–Pb data[4,10,11] for Lewisian granulites indicate that depletion of U (and by inference of Th) and alkali elements (K, Rb, Cs), continued to 2,700 Myr some 200 Myr after separation of the parent material from the mantle. The low concentrations of K, Rb, Cs, Th and U in Lewisian granulites (and possibly many other Precambrian granulites[51]) are not primary features of their igneous precursors, but are the result of metamorphic purging of these elements from the lower crust during a time interval of ~200 Myr. The characteristic depletion of alkali elements in granulites results in low Rb/Sr ratios, often lower than the bulk earth value (0.03) (refs 32, 33). This is particularly true for the Lewisian samples which have Rb/Sr ratios averaging about 0.02 (ref. 14). In contrast, amphibolite facies components of the Lewisian have Rb/Sr ratios higher than the bulk earth value. For example, the amphibolite facies gneisses studied by Moorbath *et al.*[4] have an average Rb/Sr of 0.4. The initial ^{87}Sr/^{86}Sr ratio of 0.7014 at 2,670 Myr obtained for these samples requires a time-integrated Rb/Sr ratio in the interval 2,920–2,670 Myr which is about an order of magnitude lower at ~0.03 but is still higher than that for the granulitic gneisses. Thus the amphibolitic gneisses may have acquired their high Rb/Sr ratios as a result of metamorphism and crustal differentiation. It seems feasible that some of the alkali elements lost from the granulite facies rocks during metamorphism migrated to higher structural levels.

Comparative rare-earth element (REE) abundance studies of granulite and amphibolite facies rocks[48,49] indicate that REE are not substantially fractionated by even high grade metamorphism and the data included here are in accord with these observations (see Table 2). Furthermore, the strong light REE enrichment seen in Lewisian granulites of acid to intermediate composition is compatible with their calc-alkaline nature and is not readily reconcilable with the theory that the heat producing and some other lithophile trace elements were removed in a partial melt as suggested by Fyfe[52]. Not only would such a mechanism tend to leave a residue with less fractionated rare-earth patterns than observed for most Lewisian granulite facies gneisses, but it would also tend to eradicate the isotopic evidence for earlier events. Crustal differentiation through upwards migrating non-magmatic fluids as proposed in more specific ways by Heier[6] and by Tarney and others[53] is supported by this study. On the other hand, the gross extension of such models to remove heavy rare earth and other elements from the Archaean lower crust by hydrothermal fluid transport, as recently proposed by Collerson and Fryer[54], is not supported by the Nd isotope data.

Is the time interval, $\Delta T_{\text{Nd–Pb}} = 240 \pm 90$ Myr, (during which crustal differentiation and stabilisation of granulites occurred), compatible with simple thermal considerations? To determine this we have assumed that within the interval $\Delta T_{\text{Nd–Pb}}$ the Lewisian crust can be modelled as a semi-infinite solid, containing 1 wt %K, (a conservative estimate), and $\text{K/U} = 10^4$ and $\text{Th/U} = 4$ by weight. Phase equilibrium considerations[31–33] indicate that at the end of this time interval the granulites were at a depth of at least 35 km, corresponding to a pressure of $\sim 10^4\ \text{N m}^{-2}$ (1GPa) and a temperature of ~1,100 K. If the temperature distribution in the Lewisian 2,920 Myr ago is conservatively estimated as increasing linearly with depth to 570 K at 35 km and it is further assumed that the heat input from the mantle is zero, then the subsequent temperature distribution as a function of time can be readily computed[55]. For values of the conductivity and thermal diffusivity of $3.3\ \text{W m}^{-1}\ \text{K}^{-1}$ and $1.6 \times 10^{-6}\ \text{m}^2\ \text{s}^{-1}$, respectively[56], it is found that the temperature at 35 km depth increases to 1,150 K after 50 Myr (2,870 Myr ago) and to 1,570 K after 100 Myr (2,820 Myr ago). This result indicates that extensive melting of the lower crust, even under anhydrous conditions, would occur by 2,820 Myr, unless the heat-producing elements migrated to higher structural levels

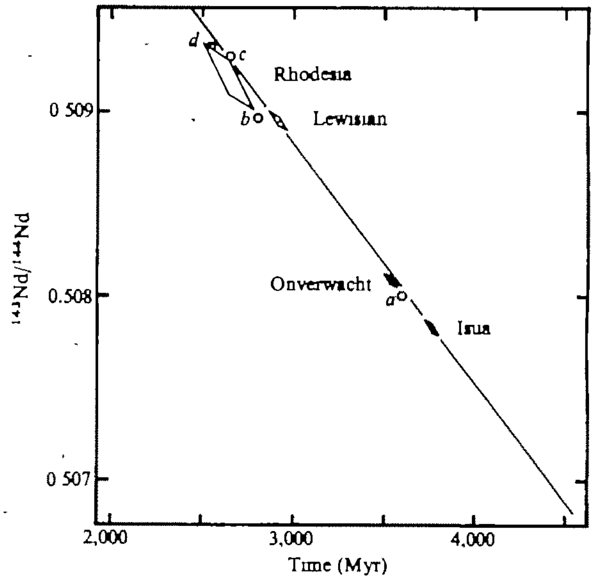

Fig. 2 Initial ^{143}Nd/^{144}Nd ratios of Archaean rocks plotted against age determined from whole-rock isochron data. Line represents growth of ^{143}Nd/^{144}Nd in a chondritic reservoir which had a ^{143}Nd/^{144}Nd ratio of 0.50682 at 4,550 Myr ago Data from DePaolo and Wasserburg[39,40], Hamilton *et al.*[17–19] and this paper. *a*, Amitsoq gneiss, Western Greenland; *b*, Preissac–Lacorne granodiorite, Superior Province, Canada; *c*, Fiskenaesset anorthosite, Western Greenland, *d*, Great Dyke, Rhodesia.

before this time. This conclusion holds for reasonable values of conductivity and thermal diffusivity and it should be emphasised that our estimates of K content and initial temperature distribution are conservative and that the mantle heat input was equated to zero. From these simple thermal considerations we may conclude that to avoid extensive melting, heat producing elements must have commenced migration from the lower crust <100 Myr after crustal thickening. The mechanism of this migration is still not clear but did not involve a transport medium which would significantly affect the Sm–Nd systematics, as would a magmatic phase.

The combined application of Sm–Nd, U–Pb and Rb–Sr systematics to ancient crustal terrains can provide important constraints on the early phases of crustal differentiation and stabilisation. This is a simple reflection of the markedly different mobilities of these elements during crustal differentiation processes. At least in the case of the Lewisian crust, the completion of metamorphic differentiation and granulite formation succeeded the primary igneous differentiation of parent materials from the mantle by 240 ± 90 Myr. The heat producing elements were transported to higher crustal levels from where heat could be readily dissipated, and the granulite facies lower crust was maintained in a more refractory and thermally stable condition.

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Pc 1 Duarte, a common polymorphism of a human brain protein, and its relationship to depressive disease and multiple sclerosis

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Pc 1 Duarte, a common variant of a human brain-specific protein, is present in one-third of the control population and has a gene frequency of 0.17. There was a significantly increased frequency of mutant heterozygotes and homozygotes among individuals with depressive disease. This is consistent with a model in which Pc 1 *Duarte is the major gene in depressive disease, acting in conjunction with an environmental threshold effect.*

THE genetic basis of the major psychoses is poorly understood. In schizophrenia, twin, family and adoption studies clearly indicate the importance of genetic factors[1–3]. The affective psychoses have been less extensively studied, but again family and twin studies provide strong evidence of genetic factors[3–5]. Proposed mechanisms for these psychoses include a single autosomal dominant gene with reduced penetrance[6–10], a single recessive gene[11], a predominantly recessive gene with an environmental threshold effect[12], polygenic inheritance[5,13–15] and in the case of manic-depressive psychosis, X-linked inheritance[16].

Several years ago I began a search for the mutant protein in Huntington disease using two-dimensional gel electrophoresis of many different extracts of brain and other tissues. Although the mutant protein in this disorder has not yet been found, a perchloric acid (PCA) extract of the caudate and putamen showed a commonly occurring mutant protein, which I call Pc 1 Duarte (ref. 17). By electrophoretic studies, it is absent from non-neural tissues and thus represents the first polymorphism of a human protein specific to nerve tissue. Because this protein is prominent in the caudate and putamen, the major site of dopaminergic neurones believed to be involved in schizophrenia[18,19], and because some genetic theories suggest a primarily

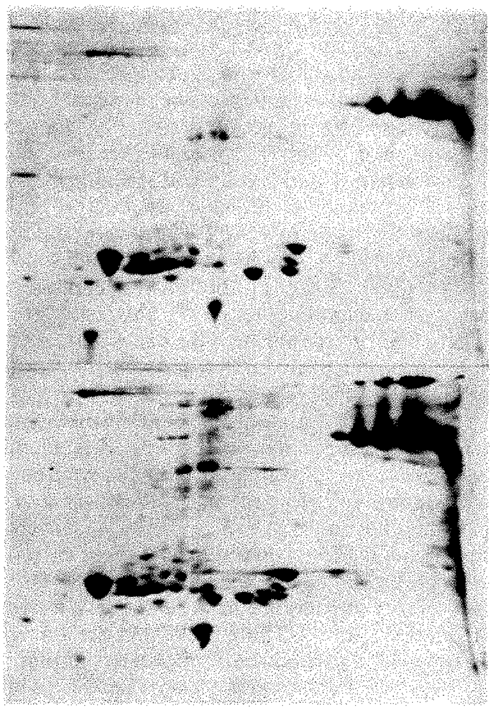


Fig. 1 Two-dimensional gel electrophoresis of PCA extracts of the caudate and putamen of homozygous normal (*Pc 1 A*/*Pc 1 A*) (top) and heterozygous (*Pc 1* Duarte/ *Pc 1 A*) (bottom) individuals. Frozen brain material was allowed to thaw and each gramme of tissue mixed with 3 ml of 0.13 M PCA plus 0.03 ml of phenylmethylsulphonyl fluoride (16 mg per ml ethanol). This was homogenised in a Teckmar homogeniser (Cincinnati) for 30–60 s. The homogenate was placed on a rocker at 4°C for 1 h then centrifuged 10,000 *g* for 20 min. The supernatant was dialysed against 0.05% β-mercaptoethanol for 4 changes then lyophilised. Electrophoresis was by the technique of O'Farrell[44] with the following modifications. Isofocusing was done in 3.7-mm i.d. Pyrex tubes with 12-cm long 5% acrylamide gel made from a stock solution of 25.5% acrylamide, 4.5% *N'N'*-diallyltartardiamide. The final gel contained 9.5 M urea, 2% NP40 and 2% BioRad ampholytes (2 parts 3–10, 6 parts 4–6, 4 parts 5–7, 1 part 7–9). The anode solution was 0.01 M phosphoric acid, the cathode solution 0.04 M NaOH, 0.02 M $Ca(OH)_2$. The gels were electrophoresed at 500 V overnight. The *p*H gradient was read directly on the gel with a flat bed electrode. The second dimension was a 12.5% acrylamide–sodium bicarbonate gel as described by O'Farrell[44]. After electrophoresis the gels were stained overnight in 0.05% Coomassie brilliant blue R dissolved in 10% acetic acid–25% isopropanol, destained for 2 h in 10% acetic acid–25% isopropanol, and destained an additional 24 h in 10% acetic acid. The gels were photographed with contrast process Ortho Kodak film with a yellow filter. To determine the molecular weight of Pc 1 Duarte, standards were included with sample in one gel and this compared with a gel without standards and with a gel of standards alone.

recessive but common mutant gene, it was of interest to determine if *Pc 1* Duarte was associated with any of the major psychoses. Significant correlations were found with the depressive diseases and with multiple sclerosis.

Electrophoresis

Two-dimensional gel electrophoresis of the PCA extract of the caudate and putamen of a normal *Pc 1 A*/*Pc 1A* (*AA*) individual and a *Pc 1* Duarte/*Pc 1 A* (*AD*) heterozygote is shown in Fig. 1. The critical portion of the gel in these two individuals plus a *Pc 1* Duarte homozygote (*DD*) are shown in Fig. 2 and the terminology is illustrated in Fig. 3. In the normal homozygote there is a major polypeptide, Pc 1 AI, and two minor satellite polypeptides, Pc 1 AII and AIII. In the variant the major polypeptide is exactly split to form an additional, more basic protein, Pc 1 Duarte I. The two minor satellite polypeptides are also split to form Pc 1 Duarte II and III. Faint polypeptides Ia and IIa migrate just ahead of both the normal and variant I and II. The splitting of three polypeptides into six in the presence of the variant indicates that AI, II and III all have the same polypeptide sequence and the separation into three forms is due to post-transcriptional modifications.

A single amino acid substitution leading to more basic mutant polypeptides explains the observed pattern. There was no correlation between this mutant pattern and drug intake, age, time from death to autopsy, or source of the brains. The patterns are highly reproducible. Many samples have been run two to three times, some seven times, with the same result.

There were approximately 20 major and 40 minor polypeptides in the PCA extracts. None of the other polypeptides were polymorphic. The Pc 1 terminology (*Perc*hloric acid extract) allows naming of Pc 2, Pc 3 and so on, as other polypeptides are studied. The protein was not present in PCA extracts of fibroblasts, liver, kidney, spleen or lung and thus seems to be nerve tissue specific.

To examine the intracellular distribution of Pc 1 A, PCA extracts of various brain fractions were electrophoresed. Pc 1 A was present only in the 100,000*g* supernatant fraction, indicating it to be a cytosol protein and not intimately attached to the nucleus, myelin, synaptosomes, mitochondria or cell membranes.

To examine the distribution of this protein, PCA extracts of many parts of the brain were electrophoresed. Pc 1 A was most prominent in the caudate and putamen, thalamus, medulla, pons and brain stem. It was present in lower relative amounts in the temporal, parietal and occipital lobe and cerebellum. The portion of the brain with the lowest relative amount was the frontal cortex. It was present in brains of stillborn infants, indicating that it was not a developmentally late protein. There was no significant difference in the amount of Pc 1 A in grey compared with white matter. Its molecular weight is 19,300 by SDS-gel electrophoresis. Its isoelectric point in 9 M urea is 5.7, comparable to 4.2–4.7 in the absence of urea.

Relation of Pc 1 Duarte to neurological disease

To determine if this mutation was related to any specific neurological disease, brains from 276 individuals who had died of various disorders were examined (Table 1). In 103 controls dying of cancer, heart disease, accident and other non-neurological disorders, there were 28 heterozygotes and 4 homozygotes from a total of 32 carrying the *Pc 1* Duarte mutant. In 49

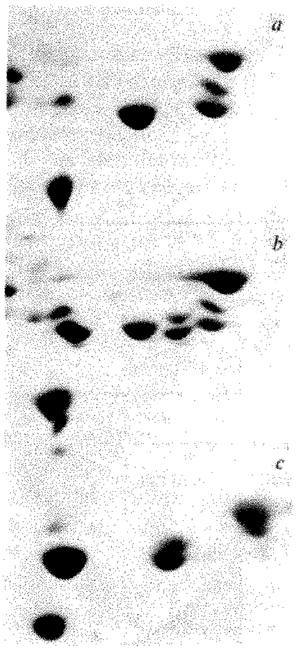


Fig. 2 Critical area of the gel *Pc 1 A*/*Pc 1 A* homozygous (*a*), *Pc 1* Duarte/*Pc 1 A* heterozygote (*b*) and *Pc 1* Duarte, *Pc 1* Duarte homozygous (*c*) individuals. In the heterozygote three polypeptides are split into six.

Table 1 *Pc 1* Duarte in various neurological disorders

Diagnosis	n	D/A	%	D/D	%	Total % carrying mutation
Controls						
Without neurological disease	103	28	27.2	4	3.9	31.1
Huntington disease	49	16	32.7	0	0.0	32.7
Total	152	44	28.9	4	2.6	31.6
Virus-associated diseases						
MS	40	21	52.5	0	0 0	52.5
SSPE	5	2		0		
AMLS	4	3		0		
Total	49	27	55.1	0	0 0	55.1
Alzheimer disease	3	1		0		33.3
Schizophrenia						
Definite schizophrenia	24	9		0		37.5
Probable schizophrenia	6	1		0		14.3
Total	30	10		0		33.3
Depressive disease						
St Louis series						
Suicide, depressive	15	6	40.0	3	20.0	60.0
Suicide, manic depressive	8	4		1		
Suicide, alcoholic	5	3		1		
Total	28	13	46.4	5	17.9	64.2
Other sources						
Suicide	3	1		0		
Manic depressive	3	3		0		
Alcoholic	8	5		0		
Total	14	9	64.3	0	0.0	64.3
Total depressive disease	42	22	52.4	5	11 9	64.3
Total manic depressive	11	7	63 6	1	9.1	72.7

No % given with n less than 10. MS, multiple sclerosis; SSPE, subacute sclerosing panencephalitis, AMLS, amyotrophic lateral sclerosis.

individuals with Huntington disease there were 16 heterozygotes and no homozygotes. As this is a single gene disorder and *Pc 1* is obviously not the affected gene, the Huntington disease patients can be added to the other controls to give a total of 152 individuals, with 48, or 31.6%, carrying the mutant gene. In these control individuals the gene frequency for the *Pc 1 A* allele, p, is $2\times AA+AD/2N$ or 0.83, and the frequency of the *Pc 1* Duarte allele, q, is 0.17. The expected DD frequency, q^2, is 0.029, which was not significantly different from the observed frequency of 0.026.

A surprising association was found with multiple sclerosis. Of 40 individuals examined, 21, or 52.5%, were heterozygotes. Comparison with the above controls by the Chi square test gives an χ^2 of 6.0 and $P<0.025$. Combination of multiple sclerosis with subacute sclerosing panencephalitis and amyotrophic lateral sclerosis, all apparently associated with viral infections[20–24], gave a total of 27 heterozygotes (55.1%) in 49 cases. Here the χ^2 was 8.77 and $P<0.005$. The relative risk[25] to *Pc 1* Duarte carriers of developing multiple sclerosis is 2.4.

The mean age of the multiple sclerosis AD heterozygotes was 50.1 ($n=20$, s.d. $=13.3$), whereas the mean age of the multiple sclerosis AA homozygotes was 47.9 ($n=18$, s.e.m. $=14.2$). These are not significantly different. Thus, it cannot be said that the presence of the *Pc 1* Duarte mutation leads to earlier, more severe disease. The frequency among 30 definite and probable schizophrenics was similar to that in controls.

Table 2 χ^2 and P values

	χ^2	P
For total % carrying the *Pc 1* Duarte mutation		
Virus-associated diseases		
Controls versus MS	6 02	<0.025
Controls versus MS + SSPE + AML	8 77	<0 005
Depressive disease		
Controls versus St Louis series	10 89	<0.001
Manic depressive disease	7.70	0 005
Controls versus total depressive disease	14 84	<0 0005
For % DD homozygotes		
Depressive disease		
Controls versus St Louis series	11 54	0.001
Controls versus total depressive disease	6 40	0.01

Because of some preliminary observations suggesting a high frequency of *Pc 1* Duarte in manic-depressive psychoses, alcoholism and suicide, an effort was made to obtain more brain samples from individuals with depressive diseases. I obtained 28 brain samples, part of a collection made over the years from individuals who had committed suicide. Medical and family records were examined and the individuals classified into suicide–alcoholic or suicide–depressive, depending on a set of criteria outlined by Feighner *et al.*[26]. Those who did not fit this classification or for whom insufficient information was available were not examined. Some of the suicide depressives were further classified as manic-depressive[26]. The results are given in Table 1. There were no significant differences between the three groups, but in the total group of 28 there were 13 AD heterozygotes and 5 DD homozygotes. Thus, in this group, 64.2% carried the *Pc 1* Duarte mutation and 17.9% were homozygous. As shown in Table 2, these figures are all significant, and for DD homozygotes in controls compared with the St Louis brains, $P=0.001$. Among suicides, manic depressives and chronic alcoholics from other sources, 9 of 14, or 64%, carried the *Pc 1* Duarte mutation. Of 42 total individuals in these groups, 52.4% were heterozygous AD and 11.9% homozygous DD for a total of 64.3% carrying the mutation. These are all significantly different from the controls (Table 2). Within the manic-depressive group only, 73% carried the *Pc 1* Duarte mutation. Based on the St Louis series, the relative risk of a DD homozygote developing depressive disease was 8.0; for the St Louis plus other series it was 5.0.

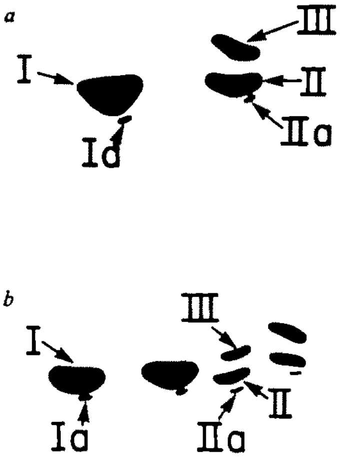


Fig. 3 Nomenclature for the Pc 1 A (*a*) and Pc 1 Duarte (*b*) polypeptides.

As the mean age of the non-Huntington disease controls was 52.6 (s.d. 18.5) and the mean age of the AD and DD individuals with depressive disease was 46.5 (s.d. 16.1), any age correction for the late onset of depressive disease would not increase the frequency of depressives among the mutant individuals.

The data on sex and race for those on whom such information was available are shown in Table 3. As this includes all cases, the total frequency for *Pc 1* Duarte is higher than that in the control group alone. The equal occurrence in males and females indicates that the gene is on an autosome. The equal occurrence in caucasians and blacks indicates that the gene has a widespread distribution and makes it unlikely that the increased frequency in depressive disease and multiple sclerosis is simply due to the presence of a subset of the population having both a high frequency of this gene and increased predisposition to these two disorders.

The genetics of depressive disease

There is a striking similarity between the results obtained here and the model of the genetics of schizophrenia proposed by Kidd

Table 3 Occurrence of *Pc 1* Duarte according to sex and race

	AA	*AD*	*DD*	Total	%*AD* +*DD*
Males	92	55 (36.4%)	4 (2.6%)	151	39.0
Females	55	37 (39.5%)	4 (4.1%)	96	42.6
Total	147	92	8	247	
Caucasians	93	59 (38.1%)	3 (1.9%)	155	40.0
Blacks	12	9 (40.9%)	1 (4.5%)	22	45.4
Total	105	68	4	177	

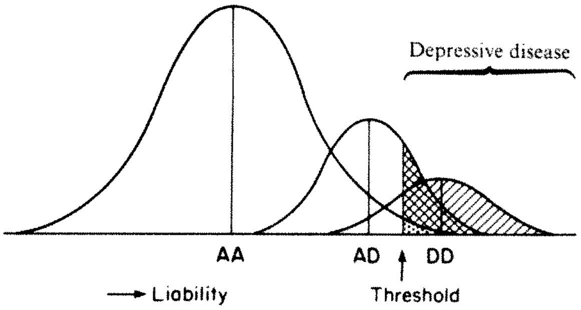
and Cavalli-Sforza[12]. Although their model was for schizophrenia, the genetic aspects of the depressive disorders are similar. In both, studies of twins show concordance rates for monozygotic twins of 30–93% and for dizygotic twins of 0–24% (refs 3–5). The significantly greater concordance rate for monozygotic compared with dizygotic twins indicates that genetic factors play an important part, and the concordance rates of less than 100% for monozygotic twins indicates that environmental factors are also important. Both have a relatively high frequency in the general population, 0.3–2.9% for schizophrenia[3] and 0.6–3.3% for depressive disease[4,27,28]. Both show a frequency in first-degree relatives of 5–39% (refs 3,4,29), significantly greater than in the general population but less than expected on the basis of a fully penetrant dominant gene.

The basic element of the Kidd–Cavalli-Sforza hypothesis is that a single, primarily recessive gene is involved in combination with a threshold effect due to environmental factors, and the gene frequency is high. I have modified their Fig. 2 to represent *AD* heterozygotes and *DD* homozygotes (Fig. 4). According to this model, *Pc 1* Duarte is the single major gene involved in depressive disease; most *AA* individuals are unaffected but some may be; *AD* individuals are at greater risk but the majority are unaffected; *DD* individuals are at greatest risk, but even here some are unaffected. The observed gene frequency is high, 0.17.

In the depressive diseases the gene would be less recessive than in the application of the model to schizophrenia. This conforms with the results of Mendlewicz and Rainer[29] in their application of the Kidd–Cavalli-Sforza hypothesis to manic-depressive disease. Depressive disease seems to be at least a partially heterogeneous disorder. The most common distinction is between monopolar (pure depressive) and bipolar (manic depressive) disease, but even here the genetic overlap is considerable[4,30–33].

Heterogeneity on the basis of response to drugs has also been suggested[34,35]. Although the incidence of *AD* and *DD* individuals may be even greater in certain types of depressive disease, the subtypes of suicide–depressive, suicide–manic depressive and suicide–alcoholic were similar (Table 1). Of the 11 manic depressives, 9 were male; the two females were *AD*. The preliminary evidence suggesting a relationship between *Pc 1* Duarte and chronic alcoholism could explain some of the genetic aspects of this disorder[36,37] and its association with affective illness[38].

Fig. 4 Application of the Kidd–Cavalli-Sforza hypothesis[12] to the present data suggesting that *Pc 1* Duarte is the single major gene involved in depressive disease and acts in conjunction with an environmental threshold effect. See text. ⊡, *AA* individuals; ▩, *AD* individuals; ▨, *DD* individuals.



Multiple sclerosis

The significant association of *Pc 1* Duarte with multiple sclerosis and with other similar diseases such as subacute sclerosing panencephalitis and amyotrophic lateral sclerosis was intriguing. These diseases are either clearly associated with viruses, as in the case of subacute sclerosing panencephalitis[21–23], or suspected of being so associated[20,24]. Studies of *HLA* genes[39–42] show an association of *HLA-A1* and *3, B7, Cw2* and *Dw2* with multiple sclerosis, the latter being the most significant. These data have suggested the presence of a multiple sclerosis susceptibility gene (*MSSG*) which is either the same as the *Dw2* gene or linked to it. Figure 5 shows the possible relationship between the *HLA* loci, *MSSG* and *Pc 1* Duarte. *Pc 1* Duarte may be linked to the *HLA* locus but separate from the *MSSG*, which is either identical to *Dw2* or linked to it (*a* and *b*). *Pc 1* Duarte itself may be the *MSSG* (*c*), or it may be unlinked to the *HLA* locus and only fortuitously associated with multiple sclerosis (*d*). Possibility *c* suggests that the presence of *Pc 1 A* Duarte may alter the host response to central nervous system viral infection. Possibilities *a* and *b* suggest that the high frequency of *Pc 1* Duarte in the population may have been due to the fortuitous occurrence of this mutation on an *HLA* haplotype that for other reasons has been selected for. This could explain the persistence of a genetically disadvantageous disease associated with a mental disorder, at a relatively high level in the population.

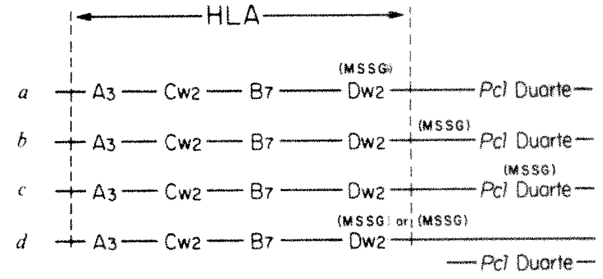


Fig. 5 Possibilities for the linkage relationship between *Pc 1* Duarte, the *HLA* locus and the multiple sclerosis susceptibility gene (*MSSG*). The *MSSG* mutation may have occurred on a chromosome carrying the *HLA A-3, Cw2, B7, Dw2* haplotype. However, these associations with multiple sclerosis do not necessarily imply that these genes are all on the same chromosome.

If the *Pc 1* Duarte mutation is the major single gene in depressive disease and is associated with multiple sclerosis, one might expect to see an association between depressive disease and multiple sclerosis. I am unaware of such an association, although Shapiro *et al.*[43] report that a significantly higher percentage of patients with a family history of affective disorders had *HLA-B7* (55%) compared to those without such a family history (19%) ($P = 0.001$). This is consistent with possibilities *a*, *b* and *c* in Fig. 5.

These studies suggest that a similar polymorphic gene interacting with environmental thresholds would account for most schizophrenia. We have examined many different subfractions of the caudate, putamen and neocortex of schizophrenics and have not yet found a comparable mutation.

The most difficult aspect of these studies is obtaining brain specimens. I would be pleased to receive frozen specimens from well documented cases of individuals dying of any behavioural or hereditary neurological disorder, especially schizophrenia, depressive disease, chronic alcoholism, hyperactivity syndrome in children, autism, tuberous sclerosis and the hereditary ataxias. These would be examined both for Pc 1 Duarte and for other mutant proteins.

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Mechanism of β-adrenergic relaxation of smooth muscle

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The mechanism of β-adrenergic relaxation was investigated in isolated smooth muscle cells. β-adrenergic agents stimulate cyclic AMP-dependent phosphorylation, enhance Na^+/K^+ transport and induce relaxation. The stimulation of Na^+/K^+ transport is obligatory for relaxation, and we suggest that this stimulation induces relaxation through enhanced Na^+/Ca^{2+} exchange.

IT has long been recognised that catecholamines cause relaxation of visceral smooth muscle[1,2]. Despite intensive studies, the biochemical and physiological events that translate this hormone signal into a change in smooth muscle tone are unknown. In other tissues, however, the events responsible for catecholamine actions are better understood. Since Sutherland and Rall suggested that cyclic AMP may serve as the intracellular mediator of the effects of glucagon and adrenaline[3,4], considerable evidence has accumulated indicating that many catecholamine actions, notably those mediated by β-adrenergic receptors[4], are mediated by protein phosphorylations catalysed by protein kinases. These protein phosphorylations are a consequence of an increase in cyclic AMP brought about by β-adrenergic stimulation of adenylate cyclase. The rise in cyclic AMP activates cyclic AMP-dependent protein kinase, which by phosphorylation of specific proteins, alters cellular functions such as glycogenolysis, lipolysis and ion transport[5–7].

An analogous sequence of protein phosphorylations has been suggested to mediate β-adrenergic-induced relaxation of smooth muscle[8,9]. As most evidence suggests that this relaxation results from a decrease in Ca^{2+} availability[10,11], it has been further suggested that the protein phosphorylated by this mechanism either directly or indirectly controls Ca^{2+} distribution[9,12]. Although this hypothesis is plausible, consideration of the literature reveals that evidence for such a cascade in β-adrenergic-induced relaxation is equivocal for several reasons[13–15]. First, an increase in cyclic AMP does not always correlate with the relaxation induced by isoprenaline. In addition, drug-induced changes in cyclic AMP-dependent protein kinase have yet to be measured. Finally, uncertainty persists regarding the manner in which β-adrenergic agents affect Ca^{2+} distribution. Studies of isoprenaline action suggest effects on the distribution of Na^+ and K^+ (refs 16–19) as well as of Ca^{2+} (refs 11, 17–23). Thus, it is not known if the effects of isoprenaline on Ca^{2+} distribution are direct or a secondary consequence of the redistribution of other ions.

The contradictions and uncertainties concerning the mechanism of β-adrenergic-induced relaxation of smooth muscle can largely be attributed to problems in interpretation of studies on intact tissue. The heterogeneity of cell types, the electrical and mechanical coupling between individual smooth muscle cells, and the complex extracellular diffusion paths have all imposed serious limitations on such studies. The availability of a preparation of enzymatically disaggregated smooth muscle cells from the stomach muscle of *Bufo marinus* permits a more direct examination of the mechanism of β-adrenergic-induced relaxation. Previous studies have revealed that these smooth muscle cells retain many of their mechanical, ultrastructural, biochemical, pharmacological and electrical properties following isolation[24–31]. Of particular importance to the present studies was the finding that isoprenaline caused a decrease in the responsiveness to contractile agents[26]. Because isoprenaline also increases cyclic AMP levels[26], we examined the possible links between these two effects of β-adrenergic stimulation. Specifically, we examined whether the link involves cyclic AMP-dependent protein phosphorylation and consequent stimulation of the Na^+/K^+ pump. In addition, we have examined how changes in the Na^+/K^+ pump are linked to changes in contractility.

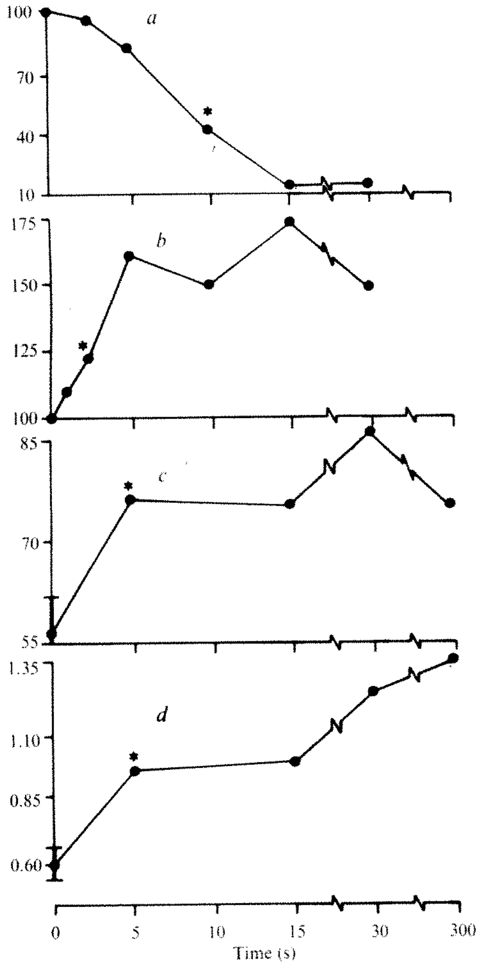

Fig. 1 Time course for the effects of isoprenaline on contractility, cyclic AMP, cyclic AMP-dependent protein kinase and phosphorylase *a* in suspensions of isolated smooth muscle cells. All experiments were carried out at 10^{-5} M isoprenaline. Cells were prepared and suspended as described previously[47]. The asterisk in all panels indicates when the response was significantly different from the control. Statistical significance for each of these four parameters was assessed from data pooled from the following experiments: *a*, % contraction, 12 experiments; *b*, cyclic AMP, 5 experiments; *c*, protein kinase, 4 experiments; *d*, phosphorylase, 4 experiments. *a*, Isoprenaline significantly decreased contractility within 10 s. Changes in the contractile state of a population of cells were assessed by comparing pulse height distributions as drug-treated and untreated aliquots of a single cell suspension were passed through a Coulter counter[47]. This method of analysing contractile changes is based on the finding that pulse amplitude is a measure of cross-sectional area that increases as the cells actively shorten. As the cells in suspension are already relaxed, the addition of inhibitory agents does not result in a shift in pulse height histograms. Therefore, we determined the changes in potential for contraction by first incubating the cells with isoprenaline and then challenging them with a brief (6 s) exposure to the excitatory agent, carbachol (5.5 μM). The effect of isoprenaline was evident as a progressive decrease in the contractile response to carbachol with longer isoprenaline exposures. It was expressed as a percentage of the contractile response observed in the presence of carbachol alone. As the effect of isoprenaline on all parameters other than contractility was determined without an excitatory agent, it is important to note that previous studies have shown that the time course for isoprenaline action on contractility is unaffected by the use of carbachol. This justifies comparison of the time course of the effects of isoprenaline on contractility determined in the presence of carbachol with the time course of effects on other parameters. *b*, Isoprenaline significantly increased cyclic AMP levels within 2.5 s. Cyclic AMP was determined by competitive protein binding assay[48] in cell extracts prepared by sonication in perchloric acid. Changes in cyclic AMP were expressed as a percentage of control cyclic AMP levels. *c*, Isoprenaline significantly increased the activity of cyclic AMP-dependent protein kinase within 5 s. Cell extracts were prepared by the addition of NaCl and EDTA to the cell suspension just before homogenisation with a Tekmar omnimixer. The ratio of protein kinase activities assayed in the absence and presence of 4 μM cyclic AMP was used as an index of the state of enzyme activation *in situ*[33]. These values are expressed as % active protein kinase. *d*, Isoprenaline significantly increased phosphorylase *a* activity within 5 s. Cell extracts were prepared by the addition of KF and EDTA to the suspension just before homogenisation with a Tekmar omnimixer and assayed as described by Hardman *et al.*[49]. Activity is expressed as nmol glucose-1-phosphate formed per min per mg protein.

Isoprenaline effects on contractility and cyclic AMP-dependent phosphorylation

A series of experiments was carried out to determine the involvement of cyclic AMP-dependent protein phosphorylation in the change in contractility induced by isoprenaline. The contractile responsiveness of the cells was monitored using the Coulter counter technique (see legend to Fig. 1 and ref. 27). As the cells in suspension were already fully extended, the relaxing effect of isoprenaline could only be assessed as inhibition of the response to an excitatory stimulus. As shown in Fig. 1, isoprenaline decreased the contractile response of isolated smooth muscle cells to excitatory agents in a time-dependent manner. It also increased cyclic AMP levels in the isolated cells (Fig. 1), and both the increase in cyclic AMP and the decrease in contractility exhibited identical dose dependencies[26]. Both effects are apparently mediated by β-adrenergic receptors, as both responses are fully blocked by propranolol (10^{-4} M). Although both responses occurred rapidly, the increase in cyclic AMP seems to occur more rapidly than the decrease in contractility. Thus, the data are consistent with the notion that relaxation is mediated by a cyclic AMP-dependent process. This is further supported by the finding that cyclic AMP mimicked the effect of isoprenaline on contractility when applied exogenously[26] or when applied intracellularly by microinjection[29].

To evaluate further the role of cyclic AMP and cyclic AMP-dependent protein phosphorylation in relaxation, we examined the effects of isoprenaline on cyclic AMP-dependent protein kinase. The toad smooth muscle contains a cyclic AMP-dependent protein kinase which, on the basis of its elution pattern from DEAE-Sephadex, is primarily the type II isozyme[32,33]. The activity of this enzyme was increased by exposure of the cells to isoprenaline (Fig. 1), with an average twofold maximal change. The increase in protein kinase activity seemed to occur more rapidly than the change in contractility. Thus, the data are consistent with the notion that relaxation is mediated by changes in the activity of cyclic AMP-dependent protein kinase.

To gain some insight into the rate at which cyclic AMP-dependent protein phosphorylation occurs in intact cells, we examined the effects of isoprenaline on phosphorylase activity. In many tissues, activation of cyclic AMP-dependent protein kinase leads to activation of phosphorylase by a sequence of protein phosphorylations involving phosphorylase *b* kinase as an intermediate. Isolated smooth muscle cells contain phosphorylase *b* kinase, as extracts catalysed the conversion of purified phosphorylase *b* to phosphorylase *a*. Exposure to isoprenaline caused a nearly twofold increase in phosphorylase *b* kinase activity (control: 0.047 ± 0.006; isoprenaline: 0.084 ± 0.0015 U phosphorylase *a* formed per min per mg protein; assayed as described by Bueding *et al.*[34]). Isoprenaline also increased phosphorylase *a* activity (Fig. 1) and dibutyryl cyclic AMP mimicked this effect. Like the activation of cyclic AMP-dependent protein kinase, activation of phosphorylase by isoprenaline occurred rapidly (significant increases were observed within 5 s). Thus, cyclic AMP-dependent protein phosphorylation can occur rapidly enough in the intact cell to mediate β-adrenergic-induced relaxation. It seemed unlikely, however, that the change in phosphorylase *per se* could account for relaxation[34]. Therefore, we investigated whether other cellular processes are altered by isoprenaline and if so, whether such alterations involve cyclic AMP-dependent protein phosphorylation.

Isoprenaline effects on Na^+ and K^+ fluxes

Several previous studies had suggested that β-adrenergic effects on smooth muscle might involve a redistribution of Na^+ and K^+ across the cell membrane[20-23]. Because of the difficulties imposed by intact tissue, these data are somewhat equivocal and inconclusive. Therefore, we investigated the effect of isoprenaline on unidirectional Na^+ and K^+ fluxes in the isolated

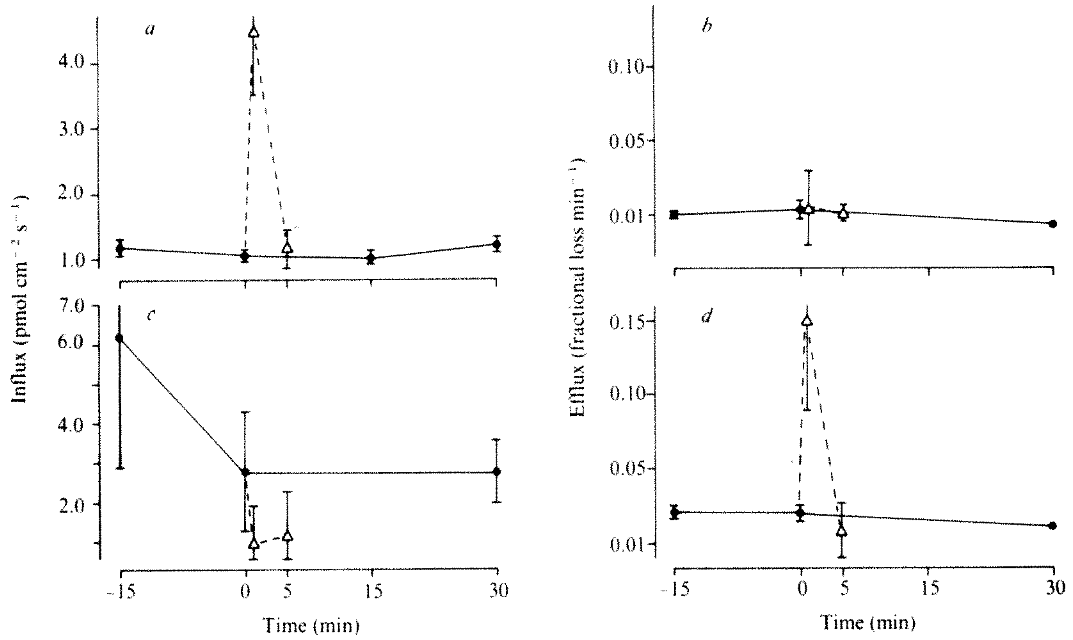

Fig. 2 Effects of isoprenaline on the influx and efflux of Na^+ and K^+ in isolated smooth muscle cells. For influx studies, cells in aliquots of a single suspension were centrifuged through silicone oil at various times after addition of radioactive ion. ^{14}C-urea and ^{3}H-inulin were included to determine the total water and extracellular water associated with the cell pellet, respectively. Cellular ion uptake (expressed as pmol cm^{-2} s^{-1}) after correction for extracellular radioactive ion, and backflux and decay of radioactive ion during counting, was calculated[50] using an average surface area/volume ratio of 5×10^3 cm^{-1} obtained by morphometric analysis of fixed cells. The same general procedure was used to study ion efflux. After several hours' exposure to radioactive ion, the specific activity of the bathing medium was reduced by dilution and the rate of loss of radioactivity from the intracellular compartment was monitored. The ion content of the cells was determined by flame photometry. An index of efflux rate was obtained by calculating[50] the fractional change in specific activity per min. In both influx and efflux experiments, isoprenaline (10^{-4} M) was added after the fluxes had achieved a stable rate, indicating exchange between a simple two-compartment system. The addition of isoprenaline was denoted time zero so that experiments could be compared. The cells did not seem to have been damaged by the experimental procedures, as the K^+ content was essentially identical to that in the intact tissue (cells: 131.5 ± 5.2 mmol per l intracellular water; tissues 138.1 ± 4.5 mmol per l intracellular water). Each point on the graph represents the mean ±s.e.m. calculated from the following experiments. ***a*, ^{42}K influx**. The addition of isoproterenol at time zero stimulated ^{42}K influx 3.4 fold (triangles) over control (circles). This effect was observed within 1 minute but was no longer evident at 5 min. ***b*, ^{42}K efflux**. The addition of isoproterenol (triangles) at time zero did not stimulate the efflux of ^{42}K above that of controls (circles). ***c*, ^{24}Na efflux**. The addition of isoproterenol (triangles) at time zero stimulated ^{24}Na efflux 7.6 fold over that of the control (circles). Like the effects of isoproterenol on ^{42}K influx, this effect was observed within 1 minute, but was no longer evident by 5 minutes. ***d*, ^{24}Na influx**. The addition of isoproterenol (triangles) at time zero did not stimulate ^{24}Na influx over control (circles). The influx values for the isoproterenol group represent an underestimate of the true values in that the backflux correction factors could not fully correct for the enhanced backflux (efflux) caused by isoproterenol.

cells. Exposure to this drug caused a large, rapid increase in ^{42}K influx and ^{24}Na efflux (Fig. 2). The stimulation was maximal at the earliest time which could be investigated (45 s) and was detectable at concentrations as low as 10^{-8} M. At concentrations of 10^{-6} M or greater, ^{42}K influx was increased by an average of 3.4 times above the control value of 1.2 ± 0.3 pmol cm^{-2} s^{-1}. ^{24}Na efflux was increased by 7.6 times above its control value of 2.9 ± 0.6 pmol cm^{-2} s^{-1}. By contrast, ^{42}K efflux and ^{24}Na influx were not significantly altered by isoprenaline. As the stimulation of ^{24}Na influx and ^{42}K efflux was no longer detectable by 5 min, large changes in ion content in response to isoprenaline are not to be expected. We calculate a net Na^+ efflux per cell in response to isoprenaline of 0.08 pmol; this represents a net decline in intracellular Na^+ of only 8%. The predicted change in K^+ content would be less than 1%. Given the analytical error associated with ion content determinations, it is not surprising that isoprenaline produced no significant change in either Na^+ (65.5 ± 4.4 mmol per l intracellular water) or K^+ (131.5 ± 5.2 mmol per l intracellular water) content. The observation that isoprenaline stimulated the movement of Na^+ and K^+ against their respective electrochemical gradients suggested that the Na^+/K^+ pump had been stimulated. To test this possibility, we examined the effects of ouabain on K^+ movement. Ouabain at 5×10^{-4} M completely abolished the stimulatory effect of isoproterenol on ^{42}K influx in three experiments; at this dose it reduced the control ^{42}K uptake by 50% (data not shown). These flux studies indicate thus that isoprenaline stimulates the Na^+/K^+ pump in the isolated smooth muscle cells. Two important questions arise from this conclusion. First, is the change in pump activity mediated by a cyclic AMP-dependent phosphorylation mechanism as has been proposed for relaxation? Second, does the change in pump activity lead to relaxation, and if so, how?

Table 1 Stimulation of Na^+/K^+-dependent ATPase activity by cyclic AMP-dependent protein kinase

	Na^+/K^+ ATPase activity (nmol per min per mg protein)
− Protein kinase	40 ± 17
+ Protein kinase	81 ± 28

Membrane fragments (50–200 μg) as described in the legend to Fig. 3 were preincubated with 10–50 μg of partially purified cyclic AMP-dependent protein kinase[51] (specific activity of 25 pmol histone phosphate formed per min per μg protein). As the protein kinase preparation had appreciable catalytic activity even in the absence of cyclic AMP, the nucleotide was not included. The reaction mixture was diluted and ATPase activity determined by the liberation of ^{32}Pi from [γ-^{32}P]ATP (ref. 52). The difference in ATPase activity assayed in the presence of K^+ alone and in the presence of $Na^+ + K^+$ was defined as Na^+/K^+ ATPase activity. Preincubation of fragments with protein kinase and $ATP\cdot Mg^{2+}$ increased Na^+/K^+ ATPase activity by an average of twofold (values above represent the mean ±s.e.m. for 8 experiments). The extent of activation was 63–180%; in 1 experiment no effect was observed. The activation of Na^+/K^+ ATPase activity was statistically significant ($P<0.05$, paired analysis). ATPase activity assayed in the absence of Na^+ was not significantly altered by protein kinase (0.194 ± 0.07 compared with 0.185 ± 0.06 μmol per min per mg). The Na^+/K^+ ATPase activity of membrane fragments exhibited several characteristics[53,54] of a Na^+/K^+ pump; inhibition by ouabain (10^{-3} M), F^- and Be^+; low apparent K_m for ATP (<50 μM); stability to mild treatment with SDS; ability to form an acid-stable, base-labile phosphoprotein; turnover number of 10^4 min^{-1} calculated from phosphoprotein and ATPase values.

Cyclic AMP-dependent stimulation of the Na^+/K^+ ATPase

A series of experiments was carried out to determine the manner in which isoprenaline affects Na^+/K^+ pump activity. The effects on the pump apparently result from interaction with a β-receptor, as the stimulation of ^{42}K influx by isoprenaline (10^{-5} M) was fully blocked by 10^{-6} M pindolol. Furthermore, the effects on the pump seem to be mediated by an increase in cyclic AMP, as dibutyryl cyclic AMP (10^{-4} M) caused an increase in ^{42}K influx identical in both magnitude and time course to that produced by isoprenaline (data not shown).

As protein kinases have been reported to affect Na$^+$/K$^+$ ATPase activity in other systems[35–38], we investigated the possibility that Na$^+$/K$^+$ ATPase may be stimulated as a consequence of cyclic AMP-dependent protein phosphorylation. Membrane fragments prepared from isolated smooth muscle cells contain an ATPase activity dependent on the presence of both Na$^+$ and K$^+$ which has characteristics similar to 'sodium pump' ATPases of other tissues (Table 1). Reacting these membrane fragments with cyclic AMP-dependent protein kinase increased ATPase activity (Table 1, Fig. 3). As seen in Fig. 3, the extent of ATPase stimulation approached a maximal value with increasing amounts of protein kinase. This stimulatory effect of protein kinase seems to result from a change in the Na$^+$/K$^+$ ATPase, as activity assayed in the absence of Na$^+$ was not affected (Table 1). Finally, the stimulation was blocked by protein kinase inhibitory protein, suggesting that the change in Na$^+$/K$^+$ ATPase specifically results from phosphorylation catalysed by the protein kinase (Fig. 3).

Na$^+$/K$^+$ transport and relaxation

Experiments were carried out to investigate the coupling between the cyclic AMP-dependent stimulation of Na$^+$/K$^+$ transport and the cyclic AMP-dependent relaxation. To assess whether these two events are causally linked, we examined the effect of isoprenaline on contractility in the presence of ouabain or in K$^+$-free medium, conditions in which Na$^+$/K$^+$ transport should be limited. In our standard medium containing 3 mM K$^+$, isoprenaline inhibited the contractile response to carbachol by 53%; however, when the cells were resuspended in nominally K$^+$-free medium just before exposure to isoprenaline, only a 16% inhibition was observed (Table 2). However, removal of external potassium had no effect on the response to carbachol. An even more striking attenuation of relaxation was observed when ouabain was used to limit Na$^+$/K$^+$ transport (Table 2).

Table 2 Effects of changes in extracellular K$^+$ and ouabain on the response to isoprenaline

Cell suspension medium	% Inhibition of contractility by isoprenaline	No of experiments
Amphibian physiological saline (APS)	50±15	3
APS+ouabain (5×10^{-4} M)	−27±32	
APS	53± 8	5
K$^+$-free APS	16±23	
APS	30± 7	5
90 mM K$^+$/APS	43±16	

The inhibitory effects of (10^{-5} M) isoprenaline on the contractile responses to carbachol (10^{-6}–10^{-4} M) were compared in aliquots of cells isolated as a group, but then divided so that half were suspended in normal amphibian physiological saline (3 mM K$^+$) and the others were suspended in modified amphibian physiological saline[25]. K$^+$ concentration changes were effected by molar substitution of K$^+$ salts for Na$^+$ salts For the groups containing ouabain or K$^+$-free medium the effect of isoprenaline was determined within 30 s of accomplishing the change from APS to the modified medium, this protocol was chosen to minimise changes in ion gradients which would eventually follow inhibition of the Na$^+$/K$^+$ pump For the cells exposed to 90 mM K$^+$, the effect of isoprenaline was determined 15 min after raising the extracellular K$^+$, this period of time was chosen to allow the cells to relax following the initial phasic contraction induced by exposure to high K$^+$ levels To test for the inhibitory effect of isoprenaline on contractility, aliquots were preincubated with isoprenaline (10^{-5} M) for 9 s before testing responsiveness to a 6-s carbachol exposure Contractility was assessed as described in Fig 1. Intracellular microelectrode recordings reveal that increasing the extracellular K$^+$ to 90 mM causes a sustained, almost complete depolarisation[31]

Thus, an increase in Na$^+$/K$^+$ pumping seems to be essential to the isoprenaline-induced relaxation.

We next considered the manner in which an increase in Na$^+$/K$^+$ pump activity might lead to a decrease in Ca^{2+} availability and thus relaxation. Two hypotheses are consistent with our present understanding of Ca^{2+} movement in smooth muscle. The first is that a decrease in calcium permeability might result from the hyperpolarisation which accompanies increased Na$^+$/K$^+$ pump activity[39–42]. Alternatively, the decrease in internal Na$^+$ generated by increased Na$^+$/K$^+$ pump activity might accelerate Na$^+_{in}$/Ca$^{2+}_{out}$ exchange in the plasma membrane and/or sarcoplasmic reticulum[43–46]. Although our understanding of the link between Na$^+$/K$^+$ pump activity and relaxation is incomplete, a change in membrane potential does not seem to be essential for isoprenaline-induced relaxation. This follows from the observation that the inhibitory effect of isoprenaline was unaffected by depolarisation of the cells with 90 mM K$^+$ (Table 2).

We are thus left with the possibility that increased Na$^+$/Ca^{2+} exchange is responsible for linking Na$^+$/K$^+$ pump activity with relaxation. Experiments in which extracellular Na$^+$ was altered suggest that Na$^+$/Ca^{2+} exchange may be important for the control of intracellular Ca^{2+} in these smooth muscle cells, as it is in other smooth muscle preparations[43–46]. Specifically, exposure of cells to Na$^+$-free medium caused contraction. The $t_{1/2}$ for this response averaged 15 min (four experiments) and the magnitude of the observed contraction was equivalent to the maximal contractile response obtained with carbachol. These data suggest that Ca^{2+} sequestration depends on the Na$^+$ gradient, and that a change in this gradient produced by stimulation of the Na$^+$/K$^+$ pump may lead to relaxation.

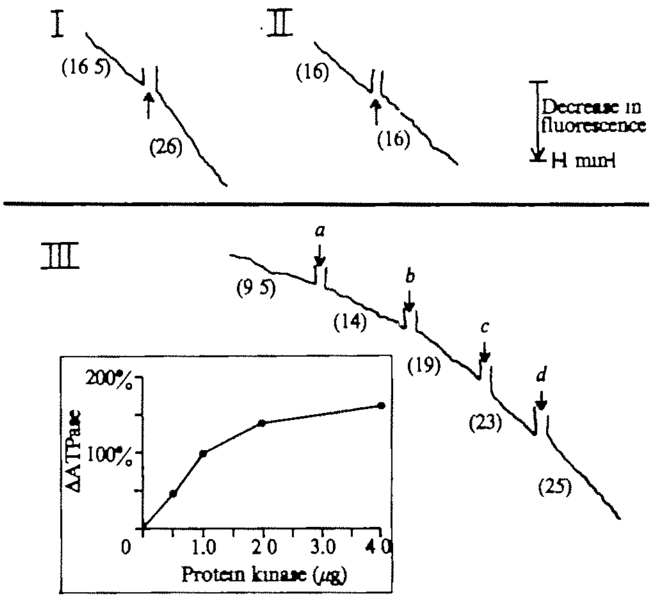

Fig. 3 Stimulation of ATP hydrolysis by cyclic AMP-dependent protein kinase. Membrane fragments were prepared from isolated smooth muscle cells by differential centrifugation of extracts prepared in sucrose (0.25 mM), imidazole (50 mM) and EDTA (10 mM) buffer, *p*H 7 2. Fragments sedimenting between 1.2×10^3 *g* and 4×10^3 *g* were assayed for ATPase activity fluorometrically[55]. ADP formation was followed as the decrease in fluorescence on conversion of NADH to its oxidised form, the rate of change being indicated in parentheses Vertical bar represents 5 nmol of NAD$^+$ In traces I and II, 10 μg of membrane fragments were used. The effects of cyclic AMP-dependent protein kinase (2 μg added at arrow) were tested in the absence (I) and presence (II) of cyclic AMP-dependent protein kinase inhibitory protein[56] added before the membrane fragments III, 20 μg of membrane fragments (a different preparation from that used in I and II) was assayed in the presence of increasing amounts of protein kinase *a*, 0 5 μg protein kinase was added, *b*, additional 0 5 μg, *c*, additional 1.0 μg; *d*, additional 2 0 μg. Note that protein kinase stimulation of ATPase activity seems to be saturable (see insert). Control studies revealed that the stimulation of ATPase activity could not be accounted for by ATPase activity of the protein kinase or by protein kinase-induced alterations in the activity of the coupling enzymes (pyruvate kinase and lactate dehydrogenase) used in the assay. It is unlikely that the stimulation of ATP hydrolysis represents a rapid turnover of a phosphoprotein in the membrane fragments catalysed by the protein kinase and a membrane-associated phosphoprotein phosphatase, as maximal catalytic activity of protein kinase could produce less than 10% of the observed stimulation of ATP hydrolysis

Model for isoprenaline action

A model for the mechanism underlying the relaxation induced by isoproterenol is shown schematically in Fig. 4. This hypothesis accounts for all of the observations on isolated smooth muscle cells. This scheme also includes what we believe to be the most plausible means by which stimulation of the Na^+/K^+ pump leads to relaxation. The essential features of the scheme are: (1) isoprenaline binds to the β-adrenergic receptor, causing a rapid increase in intracellular cyclic AMP which in turn activates cyclic AMP-dependent protein kinase; (2) the increased activity of protein kinase results in phosphorylation of a protein associated with the Na^+/K^+ ATPase, thus increasing pump activity; (3) as a consequence of increased pump activity, intracellular K^+ rises and intracellular Na^+ falls; (4) the increased Na^+ gradient would accelerate the rate of Na^+/Ca^{2+} exchange, resulting in increased Ca^{2+} efflux from the cytoplasm; (5) the change in Ca^{2+} distribution would decrease contractility.

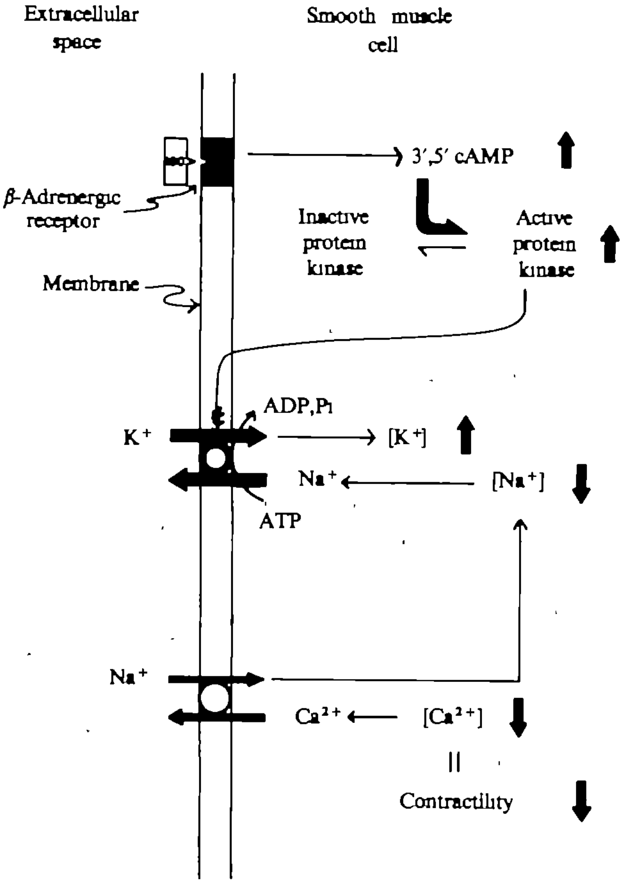

Fig. 4 Scheme for β-adrenergic relaxation of smooth muscle. It is proposed that β-adrenergic agents stimulate the Na^+/K^+ pump through cyclic AMP-dependent protein phosphorylation, and thereby produce relaxation. Although the link between enhanced pump activity and relaxation has not been fully explored, the involvement of a Na^+/Ca^{2+} exchange process seems to be most reasonable. The model shows Na^+/Ca^{2+} exchange accelerated at the plasma membrane, but a similar Na^+/Ca^{2+} exchange system in the sarcoplasmic reticulum and/or mitochondrial membranes might also contribute to the observed decrease in Ca^{2+} availability. Further details regarding this scheme and the evidence supporting are given in the text.

The above description indicates the interdependence of cellular processes which are affected by isoprenaline. Implicit in this scheme is a specific kinetic relationship between these events. First, the changes shown in Fig. 4 will be initiated in a sequential manner beginning with an increase in cyclic AMP. Second, the changes will induce a persistent activation of the Na^+ pump such that relaxation is maintained. This activation initially elicits large changes in Na^+ and K^+ fluxes, but the resultant changes in ion gradients soon slow pump activity towards basal levels. Similar kinetics for enhanced Na^+/Ca^{2+} exchange would also be expected. The large rapid increases in ion movements would rapidly bring the smooth muscle cell to a more relaxed state. Relaxation could then be maintained by only a small persistent increase in Na^+/K^+ and Na^+/Ca^{2+} transport processes.

It seems likely that the mechanism outlined above plays a central part in β-adrenergic relaxation of other types of smooth muscle, although additional factors may also be involved. Tests for the involvement of our proposed mechanism in other tissues will probably require the use of isolated cells derived from each tissue type, so that the brief, large changes in ion movements will not be obscured by the diffusion delays and ion binding which complicate studies in intact tissue.

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Structure of the backbone in myosin filaments of muscle

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New X-ray data suggest how myosin rods, themselves α-helical coiled coils, form the thick filament backbone of crustacean muscles by additional supercoiling. Natural transformations of this structure may describe the myosin backbone in many other animals also.

THE thick filaments of striated muscle are remarkable bipolar structures. The α-helical rod portions of the myosin molecules are packed densely in the backbone, yet the enzymatic portions (pairs of 'heads' or 'cross-bridges') lie at the filament surface where they can participate in the tension-generating interaction with actin[1,2]. While the ordered helical array of relaxed cross-bridges has been described for several muscles, the internal architecture that defines it is unknown: neither X-ray diffraction nor electron microscopy has revealed the structure of the backbone. Studies of the overlaps between molecules in aggregates of myosin subfragments formed *in vitro* have suggested likely packing arrangements of the myosin rods in native filaments[3–5]. Perhaps the most attractive of such models are those in which all myosin molecules have identical environments and are thus 'equivalent' to each other[6]. Knowledge of backbone structures in different muscles will help in understanding the design problem of accommodating both the structural and the enzymatic functions of myosin.

In this article, I discuss thick filament structure in the light of new X-ray diffraction data from crustacean muscles. The X-ray patterns include myosin reflections intermediate between the small-angle diffraction due to the cross-bridge array and the wide-angle diffraction from the α-helical polypeptide conformation in the backbone. These reflections suggest that the backbone has a simple cable-like structure, and indicate its relation to the surface array of bridges. Myosin filament structure in other muscles is discussed briefly in terms of plausible transformations of the structure described for the crustaceans.

Symmetry and polymorphism of the surface lattice

In the fast muscles of decapod crustaceans[7], the thick and thin filaments form a lattice resembling that in insect flight muscles, and the diffraction from the thin filaments has been discussed previously[8]. The myosin filaments are not held in precise transverse register as they are in some insect muscles, and their diffraction is unsampled. Reflections due to myosin in the small-angle X-ray pattern from a relaxed fast lobster muscle (Fig. 1*a*) include a strong meridional reflection at 145 Å with an off-meridional subsidiary maximum, and non-meridional layer lines at 308 Å and 274 Å. All these reflections become weaker in rigor, when bridges are displaced from their ordered positions round the thick filament and attach to actin. The 145-Å layer line indicates that cross-bridges are located at 145-Å intervals along the thick filament, as found for many other muscles. The non-meridional layer lines reflect the screw symmetry, or rotation between adjacent 145-Å levels (just as frog muscle patterns, for example, indicate a screw symmetry described by giving the helical repeat of the most prominent cross-bridge helix as 429 Å (ref. 9)). The screw symmetry indicated by the lobster pattern in Fig. 1*a* differs from that in muscles studied hitherto, the myosin helical repeat being 308 Å. The black spots in Fig. 3*a* illustrate this surface symmetry. The X-ray pattern is much simpler than that from frog muscles, where certain 'forbidden' reflections[9] may indicate a surface lattice in which structure units are not perfectly equivalent.

The cross-bridge reflections reveal the screw symmetry very precisely, but derivation of rotational symmetry (the number (*N*) of structure units or myosin molecules per 145-Å level) is less easy. This is because the diffraction depends mainly on the bridge conformation and on the intrinsic form of the surface lattice, and is changed only slightly by increase of *N* with proportional increase in radius from the filament axis[10]. (A preliminary study of lobster muscles[10] noted that the layer lines resemble those given by insect flight muscles[11] in intensity and

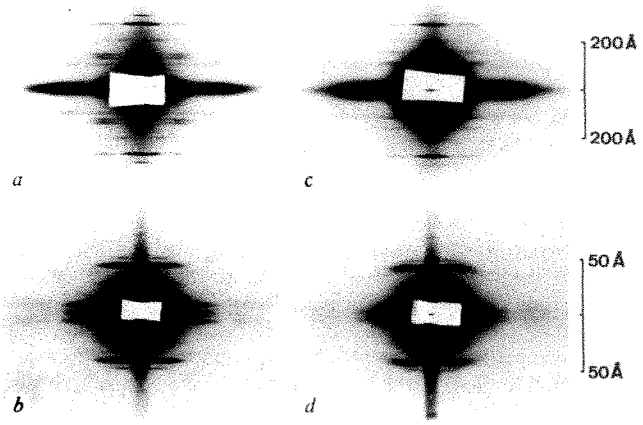

Fig. 1 X-ray diffraction patterns from crustacean muscles, chemically skinned[8] and examined in the relaxed state in a solution containing 90 mM KCl, 8 mM $MgCl_2$, 2 mM EGTA, 10 mM histidine, 2 mM Na_2ATP, 1 mM NaN_3, *p*H 7.0 at 4 °C. X-ray patterns were obtained using conventional mirror-monochromator cameras[9] and specimen–film distances of 36 cm (*a*, *c*) and 12 cm (*b*, *d*). *a*, *b*, Patterns from a fast abdominal flexor of the lobster. *a* is the small-angle pattern, showing actin layer lines at 387, 194 and 129 Å, and myosin layer lines at 308, 274 and 145 Å. The actin layer lines were enhanced, and these myosin layer lines weakened, in patterns from the same muscles in rigor. *b* is a more intense medium-angle pattern. Actin layer lines at 59 and 51 Å are prominent; the conspicuous layer line of axial spacing about 350 Å remained unchanged in the rigor pattern. Certain weak reflections in the small-angle pattern were also unchanged in rigor, such as a subsidiary maximum of the strong layer line at 380 Å and a layer line at 95 Å. *c*, *d*, Patterns from a slow muscle of the lobster crusher claw. Slow muscles gave patterns with a higher diffuse background than fast muscles, but layer lines corresponding to those from fast muscles were clearly visible. The small-angle off-meridional reflections occurred at 350 and 248 Å, and the myosin layer line which was unchanged in rigor fell at about 600 Å.

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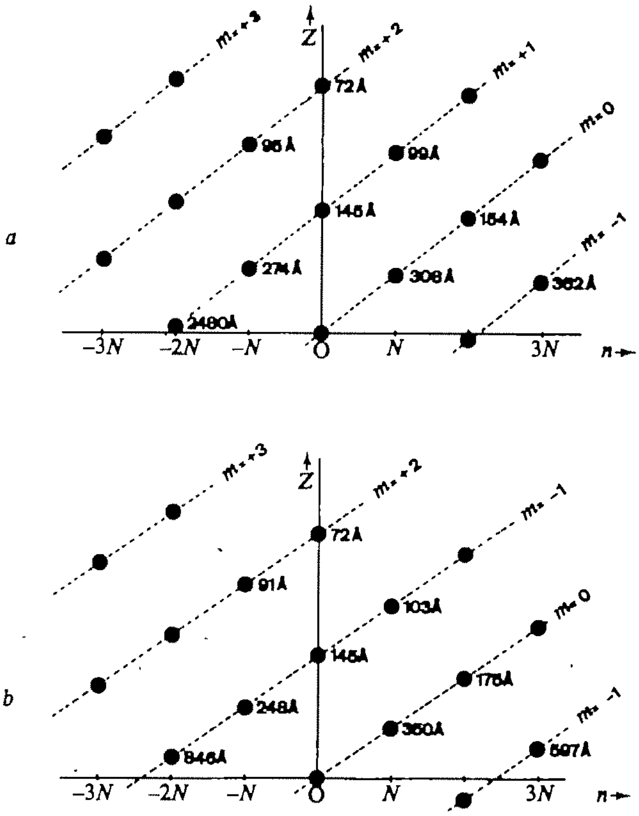

Fig. 2 Diagrams of myosin layer lines *a*, General representation of the diffraction expected from a helical array with the symmetry being indicated by the myosin diffraction in Fig. 1*a*. Axial positions (Z) of expected layer lines are plotted against their Bessel function orders (n), reflections can be referred to by n and by the value of m assigned to the dotted lines (in the convention of helix diffraction theory[23]), and certain axial spacings are indicated numerically Individual nodes are represented in the observed diffraction by reflections whose radial positions depend on the radii from the filament axis of the structures generating them. The diffraction in Fig. 1*a* is interpreted through the diagram as follows The strong layer lines at 308, 274 and 145 Å originate from the surface array of cross-bridges, and generate the innermost reflections ($m=0$, $n=N$), ($m=1$, $n=-N$) and ($m=1$, $n=0$) respectively. Bridges do not contribute significantly to any wider-angle reflections, which correspond to other sets of helical tracks through the surface lattice. The reflection ($-1, 3N$) at 352 Å has special significance, as a strong layer line at this spacing is due to subfilaments interconnecting the surface lattice; the value $n=3N$ corresponds to there being $3N$ subfilaments. (The backbone is not formed from smooth strands; other variations of density, distributed in conformity with the myosin surface lattice, cause weak diffraction at other nodes, such as a subsidiary peak on the 308 Å layer line ($0, N$) and presumably part of the meridional intensities at orders of 145 Å.) Many reflections thus include contributions from both bridges and backbone, located at different radii from the filament axis, and only in special cases will profiles be interpretable in terms of diffraction from simple structures. *b*, Same diagram for the slow muscles. N here takes a different value. These diagrams represent two stages in the continuous transformation of the diffraction that would accompany progressive twisting of the filament. The various fast crustacean muscles could be represented by a series of diagrams with spacings close to those in (*a*) The spacing of the ($-1, 3N$) reflection equals the helical repeat of the subfilaments in each case. Generally, such diagrams can describe the diffraction from any helical cross-bridge array. Untwisting the filament slightly beyond state (*b*), the spacing of ($0, N$) reaches 385 Å as observed for insect muscle[11], and ($-1, 3N$) then has a spacing of 1150 Å. Untwisting further, until ($0, N$) falls at 435 Å, the screw symmetry becomes that observed for frog muscles[9]. ($-1, 3N$) lies on the equator in the latter case: thus, if the filament were formed from subfilaments homologous to those of crustacean muscles, these would run parallel to the filament axis.

might be explained by the sixfold rotational symmetry widely accepted for insect muscle: but, as discussed below, this symmetry for lobster muscles now seems unlikely.) Neither the value of N nor the conformation of each pair of heads is required for discussing the relationship between myosin symmetry and the structure of the backbone.

The myosin screw symmetry in fast crustacean muscles depends on the anatomical location of the muscle and on species. For example, the principal cross-bridge helical repeat was 300 Å in many fast muscles of crayfish and 308 Å in certain fast lobster muscles, but 310 Å in the fast muscles of crayfish legs, 315 Å in those of the lobster left claw, and 318 Å in those of both crayfish claws. Thus, while myosin filaments may have the same screw symmetry in the skeletal muscles of all vertebrate animals, the fast muscles even of one crustacean species present a family of non-integral myosin helices differing slightly in filament twist.

Diffraction from the myosin backbone

The diffraction expected from a representative member of this family is summarised in Fig. 2*a*. The observed cross-bridge reflections (at 308 Å, 276 Å and 145 Å) represent few of the full set that would result from a perfectly ordered helical array, and this paucity of diffraction indicates that the bridges are considerably disordered about their average positions. More intense patterns from the crustacean muscles did show other myosin reflections, but these were not due to bridges, as they were unchanged in patterns from rigor muscles. The most prominent was a layer line (Fig. 1*b*) whose spacing varied with the screw symmetry of the filament so as always to correspond to that of one particular outer reflection (at 352 Å in the case depicted by Fig. 2*a*). The characteristic radial profile of this layer line is shown in Fig. 4*a*.

Reflections unaffected by the transition to rigor but occurring at spacings related to those of the myosin surface lattice are expected from the myosin backbone and may reveal its structure. First, as different nodes in Fig. 2 correspond to different sets of helical tracks through the surface lattice, the prominence of one particular layer line means that the structure can, at low resolution, be considered as comprising uniform helical strands following one particular direction. Second, the profile of this layer line is characteristic of a cable-like structure whose density is concentrated near a single radius, and whose strands have a diameter of about 40 Å and are packed closely with their neighbours (Fig. 4*b*). The backbone is shown in Fig. 3*a* as a cable whose strands connect up the cross-bridge surface lattice in the direction corresponding to the prominent layer line from the backbone. This structure, apparently identical in relaxed and rigor states, could exist over most of each half of the myosin filament, but will not describe the packing at the ends and centre. Its absolute hand is unknown and is drawn arbitrarily. The hollow core is in agreement with the appearance of a weakly staining centre in crustacean filaments[7]; this observed appearance is variable, perhaps because the core contains small but variable amounts of paramyosin (whose organisation is not revealed by the present X-ray data).

The relationship of cross-bridge positions to the backbone in Fig. 3*a* is such that, with N myosin molecules per 145 Å along the filament, there are $3N$ subfilaments. (The value $N=4$, as drawn, seems the most probable if the tubular appearance of Fig. 3*a* is identified with that in electron microscope images, because the number ($3N$) of subfilaments, separated centre-to-centre by 40 Å as indicated by the X-ray pattern, must be about 12 to generate the observed[7] outer diameter of about 200 Å.) The X-ray patterns also contain information about the packing of myosin rods within individual subfilaments. But the 40-Å subfilament diameter directly suggests a cylindrical group of three myosin rods (each a 2-stranded α-helical coiled coil). Moreover, the repeat length along each individual subfilament in Fig. 3*a* is 435 Å, so the length of the crustacean myosin rod[12], slightly more than 3 × 435 Å, would likewise suggest that three overlapping rods comprise each subfilament over most of its length. The myosin rods forming each subfilament must twist round each other if all the myosin heads are accessible to the filament surface.

Diversity of crustacean filaments

The variation of screw symmetry was the only indication in the X-ray patterns that myosin structure in fast crustacean muscles is polymorphic. It corresponds to a slight variation in the angle at which the subfilaments twist round the filament. The versatility of the packing is further illustrated by the slow muscles of the crustaceans. Here the thick filaments have a larger (280 Å) diameter[7], reflected in the X-ray pattern (Fig. 1*c*) by, for example, a different profile of the 145-Å layer line. But although the two off-meridional cross-bridge reflections indicate a cross-bridge helical repeat of 350 Å, and the layer line from the subfilaments has an axial spacing of about 600 Å (Fig. 1*d*), the intensities and profiles of these layer lines are not greatly changed from those in the several fast muscles. Thus an essentially invariant relationship of relaxed cross-bridges to backbone subfilaments seems to be intrinsic in the surface lattice of the fast and slow muscle myosin filaments. The slow muscles differ merely in a smaller twist of the subfilaments round the filament, and in higher rotational symmetry (probably increased from four- to fivefold (Fig. 3*b*)).

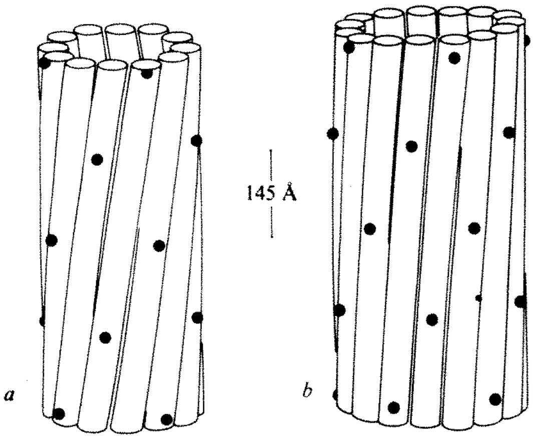

Fig. 3 Relationship of cross-bridge positions to subfilament organisation. *a*, Black spots represent the positions of cross-bridges, as indicated by those reflections in Fig. 1*a* which weaken in rigor. Further aspects of bridge arrangement, such as radial position and conformation, are not discussed here. The backbone is shown as strands connecting this lattice in the helical direction corresponding to the prominent backbone layer line; subfilament diameter and separation were derived from this layer line (Fig. 4). The several fast crustacean muscles examined would have uniform rotational symmetry, but slightly different screw symmetries. *b*, Similar drawing for slow muscle myosin filaments. Both the rotational and the screw symmetry differ from (*a*). The essentially conserved relationship between bridges and subfilaments probably extends to finer details of the structure than are indicated here.

Subfilament structure of myosin filaments

These models for myosin filaments are of a type discussed by Cohen[6]. Re-emphasising that the filaments might be constructed so that all myosin molecules are equivalent, Squire[5,13] has enumerated such packing arrangements more fully. The fast and slow muscles of crustaceans illustrate a central point of Squire's general thesis: that whatever the local packing relationships of myosin rods, the unit cell of the surface lattice may change little if the cross-bridge arrangements of different muscles are generated by changes of rotational and screw symmetry. The particular packing of rods discussed by Squire[14] as a model for all myosin filaments is more compact than the present structure: groups of rods do not twist round each other in subfilaments near the surface, but instead form radial sheets which more nearly fill the filament centre. The present X-ray data require a backbone organised so that there is a marked circumferential repeat of 40 Å rather than the less 'contrasty' surface appearance of the model favoured by Squire[14].

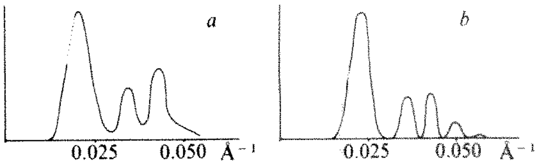

Fig. 4 *a*, Observed radial profile of the strong layer line at 352 Å, indexed as $(-1, 3N)$ in Fig. 2*a*. Dashed line indicates uncertainty due to incomplete separation from nearby layer lines. *b*, Intensity calculated[24] for the layer line at 352 Å from the cable-like backbone in Fig. 3*a*, normalised to same peak height as (*a*). Subfilaments, centred at 84 Å radius, were each represented by 3 uniformly intertwining myosin rods (smooth cylinders of 8.5 Å radius) centred 12.6 Å from their local axis. The 352-Å layer line is the 'pitch' layer line of the 'major helix'[24], and is by far the strongest of the layer lines from the structure. In terms of Bessel functions (J), the profile is given by

$$[J_{12}(2\pi \times 84R)J_0(2\pi \times 12.6R)J_1(2\pi \times 8.5R)/(2\pi \times 8.5R)]^2$$

Cables with more or fewer subfilaments, but with the same (~40 Å) separation between neighbours, give similar profiles: but the 12-fold symmetry appears the most probable (see text).

There are two reasons why X-ray patterns from crustacean muscles are especially informative about backbone structure. First, the pronounced twisting of the subfilaments round the filament means that a key diagnostic layer line can be observed, separated from the equator of the X-ray pattern. Second, the distinct but related filament structures in the various crustacean muscles indicate the conserved and the variable aspects of the molecular packing. Corresponding data from other animals will establish how far myosin backbones in muscles outside this restricted class can be described by the present model. X-ray data on cross-bridge arrays in some invertebrate muscles apart from crustaceans (such as *Lethocerus* flight muscle[13] and *Limulus* muscle[15]) can be interpreted in terms of a simple helical surface lattice: backbone models for these filaments must again be such that myosin molecules occupy equivalent positions. In fibrillar insect flight muscles, for example, the 385-Å myosin helical repeat[11] may result from further change of screw symmetry in a filament of the crustacean type, as a reflection with the axial spacing of roughly 1,150 Å, as expected for diffraction from subfilaments (see legend to Fig. 2), is observed at 40 Å radius (my unpublished results on bee and fly muscles, and ref. 16). The flight muscles of some dragonflies (Odonata) are non-fibrillar, and support this extension of the crustacean model to include insect filaments. Their myosin symmetry differs from that in the fibrillar muscles: the helical repeat is about 350 Å, and the subfilament diffraction is very like that of slow crustacean muscles. Application of the subfilament model to insect as well as to crustacean filaments does not demand the same rotational symmetry in both cases, but does leave the puzzle that, just as argued above for crustaceans, the 150–200-Å diameter of insect filaments observed by electron microscopy[17] could accommodate 12 subfilaments, and thus 4 myosin molecules per 145 Å of filament length, while other evidence (see ref. 18) suggests 6 myosins per 145 Å for insect muscle.

Vertebrate and other filaments

In some other muscles, such as vertebrate striated muscles[9] and the striated adductor of the scallop[10,19], X-ray evidence suggests that the cross-bridge array departs from equivalence in that adjacent 145-Å levels are not identical (possibly because of non-equivalent packing in the backbone or a perturbation due, for example, to proteins other than myosin). Here I consider non-equivalence only in the context of the present subfilament model, retaining the 435-Å repeat within the subfilament and the 145-Å stagger between subfilaments as conserved features. Filaments built in this way provide equivalent environments for all myosin molecules only when each subfilament, at every level at which cross-bridges emerge from it, appears at the filament surface in an identical way. Disposition of the subfilaments to form tubular filaments in crustaceans fulfills this requirement in a simple manner (which, however, leaves a large core). But strict equivalence is not necessarily a significant property for the cross-bridge array: the departures detected by X-ray diffraction may be rather small, and may leave all myosin molecules functionally equivalent in their ability to interact with actin. More compact structures would result from an orderly collapse of the tubular form (with loss of perfect helical symmetry, as cross-bridges from more central subfilaments would necessarily adopt slightly different resting configurations). Collapse is plausible only when the subfilaments run almost parallel to the filament axis (as inner and outer subfilaments otherwise acquire different axial repeats), or when the filament is very short, but could in either of these cases occur with little change in the pattern of intermolecular connections. Vertebrate skeletal myosin filaments might exemplify this more radical transformation of the crustacean structure, as their screw symmetry is such that the subfilaments would run parallel to the axis (see legend to Fig. 2). Collapse could give solid polygonal filaments as observed for frog and fish muscles[20,21]. The consequent non-equivalence would be additional to that resulting because C-protein is not located according to the 143-Å repeat of myosin[22]. Such a model for vertebrate filaments remains entirely speculative until direct information about their backbone structure is obtained, but meanwhile illustrates one natural way for more specialised myosin structures to be generated from a simple, versatile and widespread design.

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letters

Are there organic grains in the interstellar medium?

THE possibility that organic compounds exist in or on interstellar grains has received much attention recently. It has been suggested that grains consist of polysaccharides[1], carbonaceous compounds[2] or aggregates of a variety of organic compounds[3]. While it is easy to visualise how some of these materials could form in the interstellar medium (for example, by the random accretion of C, N, O and H) it is not certain that appreciable quantities of such grains actually exist. With the availability of high quality infrared (IR) spectra of a variety of sources we show here how to determine the fraction of interstellar carbon bound in complex molecules of this sort.

Virtually all compounds containing C and H possess an IR absorption band due to the excitation of the C–H stretching vibration between 3.3 and 3.4 μm. This band usually has a width of ≃0.05–0.2 μm in condensed phases. As noted by Knacke[2] this band will be present even in polymeric or tarry materials as found, for example, in carbonaceous chondrites. The persistence of a band at 3.3–3.4 μm in all organic systems implies that observations of the spectrum of interstellar dust in this region can be used in a determination of the abundance of organic solids in interstellar dust.

Figure 1 shows a plot of τ(3.3–3.4 μm) against A_v, the visual extinction in magnitudes, for a variety of organic compounds representative of several chemical classes. These optical depths at 3.3–3.4 μm would be obtained if all interstellar C were obtained in these molecules. They are calculated on the basis of an extinction $A_v = 1.6$ mag per kpc H atom cm^{-3} using the normal cosmic abundance of carbon[4]. Data on the strength of the 3.3–3.4 μm band was obtained from standard sources[5,6]. Cross-sections vary from a low of 3.4×10^2 cm^2 g^{-1} for C_6H_6 to 8.4×10^3 cm^2 g^{-1} for $C_{42}H_{88}$ (2,2,4,15,17,17-hexamethyl-7,12-di(3,3,5-trimethyl hexyl)octadecane). It can be seen that the largest values of τ are produced by alkanes. The cyclic ether, propylene oxide also produces strong absorption at 3.3–3.4 μm. Aromatic compounds such as C_6H_6 are relatively weak absorbers although substitution with any CH bearing group (such as *p*-xylene) greatly increases the 3.3–3.4 μm band strength. Polymers such as polyethylene and polyvinyl alcohol are strong absorbers.

The corresponding relationship between equivalent width, W, in μm and A_v is

$$\frac{W}{A_v} = 2.9 \times 10^{-2} \frac{I}{n_c} \tag{1}$$

where I = integrated 3.3–3.4 μm band intensity in units of 10^4 l cm^{-2} mol^{-1} and n_c = number of carbon atoms in each

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absorbing molecule. This equation is obtained from Wexler's expression[6] for integrated intensity in these units, $I = \Delta\nu \log_{10}(\mathcal{I}_0/\mathcal{I})/(10^4 CL)$ where $\Delta\nu$ is FWHM if cm^{-1}, $\mathcal{I}/\mathcal{I}_0 = e^{-\tau}$ is the intensity ratio in the line, C is the concentration in moles per 10^4 litre, and L is the path length in cm. Most bands have intensities, in these units, in the range 0.01–1, (ref. 6). For small τ, $W = \tau\Delta\lambda$, and so rearranging, with some attention to units, gives $W = N\lambda^2 I/2.62\times10^{20}$. W and λ are in μm, and N is the column density (cm^{-2}) of absorbing molecules. Thus $N = [C]L/n_0$ where $[C]$ is the total available carbon density (cm^{-3}). With extinction A_v of 1.6 mag kpc^{-1} per H-atom, then $A_v = 1.6[\text{H}]L/3\times10^{21}$. Using the cosmic abundance of carbon, the ratio of W to A_v gives equation (1). Since $I \simeq n_0 G$ where G = integrated intensity per structural unit (CH, CH_2 or CH_3),

$$W \simeq 2.9\times10^{-2} A_v G \qquad (2)$$

G is quite different for molecules of different chemical classes but is relatively constant for molecules of the same class[6]. Figure 1 shows data for a selection of molecules that are representative of the major chemical classes. Obviously under interstellar conditions a variety of molecules would be expected and so an average would have to be taken to determine the total expected 3.3–3.4 μm optical depth.

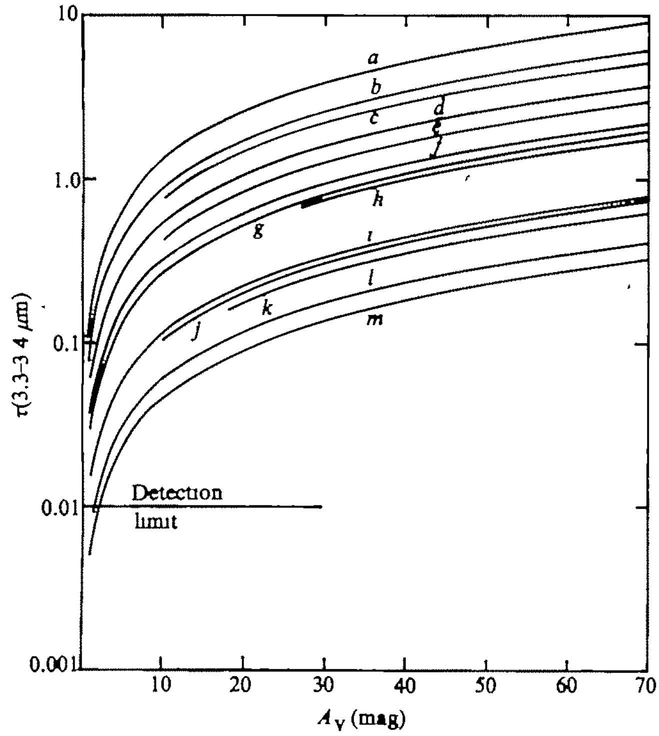

Fig. 1 Predicted optical depth at 3.3–3.4 μm, τ due to organic compounds on grains plotted against visual extinction A_v in magnitudes. A detection limit provided by recent observations[7] is shown at $\tau = 0.01$. Failure to detect the presence of a 3.3–3.4 μm band with this amplitude in an object with $A_v = 70$ mag would imply that < 0.2% of interstellar carbon is present as alkanes: *a*, $C_{42}H_{88}$; *b*, *n*-hexane; *c*, propylene oxide; *d*, 1-pentanol; *e*, *n*-butyl ethyl ether; *f*, polyethylene, *g*, acetone, *h*, poly-vinyl alcohol; *i*, *p*-xylene; *j*, 1–3 butadiene, *k*, pyridine; *l*, 3-octyne; *m*, benzene.

A line in Fig. 1 indicates the current detection limit for spectral features in the 2.9–3.4 μm region as obtained, for example, by McMillan[7]. Spectroscopic data of high quality obtained by Merrill *et al.*[8] for a variety of highly obscured objects shows no evidence for detectable 3.3–3.4 μm absorption even in objects with $A_v \gtrsim 50$ mag. It is also significant that no absorption appears at 3.3–3.4 μm even in objects with well developed 3.08 μm ice bands. These observations, together with the curves given in the Fig. 1 suggest that the limits shown in Table 1 can be placed on the abundance of organic compounds on dust in the interstellar medium.

Table 1 Abundance limits of various chemicals in the interstellar medium

Chemical class	Abundance limit
Alkanes	<1% of C
Cyclic ethers	<1%
Non-cyclic ethers	<2%
Alcohols	<2%
Polymers	<2%
Ketones	<2%
Alkenes	<10%
Alkynes	<10%
Aromatics	<10%

These limits are expressed in terms of the percentage of carbon that could be present in molecules of each class without producing $\tau(3.3\text{–}3.4) > 0.05$ for $A_v = 70$ mag. As all molecules absorb at the same wavelength it is not possible to sum abundance limits in Table 1 to permit the dispersal of larger amounts of carbon in organic compounds. Thus if 1% of C were in alkanes, $\tau = 0.05$ would be produced by these molecules alone implying that the abundances of all other types of organic molecule would be much less than those given in Table 1.

We conclude that no spectroscopic evidence exists to support the contention that much of interstellar dust consists of organic materials. While the presence of trace quantities of organic compounds on grains inside very dense clouds cannot be excluded by available data, the absence of any observation of a 3.3–3.4 μm absorption band even in objects with $A_v \simeq 50$ mag strongly suggests that organic grains constitute at most a minor component of interstellar dust and probably have no observable effect on visual extinction. Certainly the dust that produces the visual extinction in highly obscured sources cannot be composed of organic material. For example, in an object with $A_v = 50$ mag the column density of dust in the form of particles of radius 10^{-5} cm with scattering efficiency $\simeq 1$, is $(NL)_{\text{dust}} = 1.6\times10^{11}\ \text{cm}^{-2}$. For a grain density of 1 g cm^{-3} the mass of dust per cm^2 is 6.4×10^{-4} g cm^{-2}. Then $\tau(3.3\text{–}3.4)\ C_6H_6 = 0.22$ and $\tau(3.3\text{–}3.4)\ C_{42}H_{88} = 5.4$ in this object. Optical depths in this range would certainly be detectable.

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Magnetic buoyancy in the Sun

THE concept of 'magnetic buoyancy' was introduced[1] as a possible mechanism by which magnetic fields from within the Sun might come to the surface and contribute to its magnetic activity. We discuss this concept here and consider its effect on solar dynamics.

An isolated flux tube of field strength **B** in a gaseous atmosphère of magnetic permeability μ has a lower thermodynamic pressure p than that of its surroundings, because the total

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pressure $p+\frac{1}{2}\mu^{-1}\mathbf{B}^2$ must be the same inside and outside the tube. If the temperatures inside and out are sufficiently close it will then be lighter than its surroundings and will tend to rise by this magnetically-induced buoyancy.

Recently[2,3] it has been argued that this mechanism actually works rather too well for comfort, bringing 100-G flux tubes from all but the bottom quarter of the solar convection zone (depth ~200,000 km) to the surface in a good deal less than the time needed for the generation of these fields by the hydrodynamic dynamo process[4], which is 10 yr, that is, comparable with the time scale of the solar cycle. As far as the origin of these thin flux tubes is concerned, it has been pointed out that a large-scale azimuthal field which decreases with height is unstable[3], again by the mechanism of magnetic buoyancy, and would break up in the required way within a time of order H/V, where H is the scale height for the density ρ and

$$V=B/(\mu\rho)^{1/2} \tag{1}$$

is the Alfvén speed. This 'break-up' time is also much less than 10 yr except at the very bottom of the convection zone. The conclusion of this argument is that the dynamo must function at the very bottom of the zone with a field strength of ~100 G; such a field is barely enough to account for the total flux emerging from the Sun in the course of one solar cycle[3].

As flux tubes rise they encounter lower background pressures and if they are horizontal they expand in cross-section. Their magnetic field therefore drops, and it has been argued[5] that the concomitant reduction in their magnetic buoyancy can substantially increase the time they take to reach the solar surface. The matter is complicated, however, by the fact that by bending, so that some portions are higher than others, the 'crests' can largely retain their magnetic buoyancy by off-loading[1] some of the gas down the arms of the tube to the 'troughs', which proceed upwards rather more slowly as a result. The initial instability mechanism certainly uses this process, so that the most rapidly growing disturbances have non-zero wavelength (in fact, comparable with the magnetic field scale-height) along field lines[6,7], and it is natural to expect this bending of the field lines to persist even when the amplitude of the disturbance becomes large.

Further bending of the tube, of a different kind, will result from the action of the Coriolis force (due to the Sun's angular velocity Ω) on the material sliding down from the crests to the troughs; this imparts an anticyclonic twist ψ to the crests, which as a result do not break the surface quite along a line of latitude. This is thought to explain the fact that the 'leading' sunspot of a bipolar pair is normally closer to the Equator than the 'following' spot[4]. The observations demand that typically $\psi\sim 10°$.

Recent studies of instability by magnetic buoyancy in a rotating system[8-10], however, suggest the solar rotation exerts a much stronger influence than this on the entire mechanism: the dimensionless parameter

$$S=\frac{\Omega H}{V} \tag{2}$$

is large throughout most of the convection zone for an azimuthal field of 100 G, ranging from ~20 at a depth of 30,000 km to ~500 at the bottom of the zone (~200,000 km depth). It turns out that magnetic buoyancy instability does not then develop on the Alfvén time scale H/V at all, but on the much longer time scale

$$T\sim\frac{4\Omega H^2}{V^2} \tag{3}$$

This is ~10 yr at 30,000 km depth, again for a field of 100 G, but far exceeds the time scale of the solar cycle at greater depth. Further, if the effects of turbulent diffusion are taken into account instability does not take place at all unless

$$\mathscr{C}=\frac{V^2}{2\Omega\eta} \tag{4}$$

is greater than about unity. With turbulent diffusivities η of the kind usually envisaged[11,12], that is 10^8–$10^9\ \mathrm{m^2\,s^{-1}}$, $\mathscr{C}$ is ~10^{-3} or so throughout much of the zone.

Suppose, therefore, that the azimuthal field were much larger, say at least 1,000 G. The parameter $\mathscr{C}$ might just about exceed unity (η is somewhat uncertain[12]), while the time scale T ranges from a few weeks at depths of 30,000 km to ~10 yr at 100,000 km. In the bottom half of the convection zone we would not expect even a 1,000-G field to break up by magnetic buoyancy instability within the time scale of the solar cycle.

The most rapidly-growing mode has a wavelength along field lines of order H, as in the absence of rotation, and the original field again breaks up into isolated flux tubes with 'crests and troughs'. What happens to these tubes when they have floated up and distorted a significant amount is beyond the scope of the linear instability theory, but it seems natural to suppose that the rate of rise will then be comparable with the actual gas velocities $\mathbf{u}$ involved, and that the time scale will be comparable with that for the instability. Then the rate of rise and $\mathbf{u}$ will be of the order $V^2/\Omega H$, which is much smaller than the Alfvén speed, and implies that the Coriolis forces (per unit mass) $2\rho\boldsymbol{\Omega}\wedge\mathbf{u}$ and the Lorentz forces $\mu^{-1}(\nabla\wedge\mathbf{B})\wedge\mathbf{B}$ will be of comparable magnitude. This avoids a contradiction that arises if one assumes that rotation plays only a rather minor role and that the rate of rise and $\mathbf{u}$ are of order V (see above): the ratio of Coriolis forces to Lorentz forces is then $\Omega H/V$ and very large (for a 100 G field), indicating that rotation must, in reality, have had a crucial effect on the motion of the tube.

Consideration of magnetic buoyancy may place restrictions on solar dynamics which are rather different to those which have recently been suggested, because the rotation of the Sun has a very strong influence on the mechanism. Fields of 1,000 G might be retained in the middle of the zone for 10 yr or so; deeper down even stronger fields could be retained. If magnetic buoyancy is responsible for bringing to the surface the fields that are observed, their origin must be strong ($\gg$1,000 G) azimuthal fields in the top half of the convection zone. Such fields would make it easier to account for the total flux emerging from the Sun in the course of a solar cycle[3], and would also give the rising flux tubes more resistance to Coriolis twisting, which together with a shorter travel distance to the surface (compared with the full depth of the convection zone) would help keep ψ at a relatively small value.

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Possible use of ^{41}Ca for radioactive dating

A LARGE number of cosmogenic isotopes have been suggested for use in radiometric dating[1]. One which has not received much attention is ^{41}Ca (half life $t_{1/2}=1.3\times10^5$ yr (ref. 2)). This is probably due to its small expected concentration in natural samples and the difficulty in detecting its pure electron capture decay. On the other hand, the importance of calcium in many geochemical and biochemical cycles ensures that any method of dating this element would have widespread application. Recent important developments in the detection of radioisotopes using accelerators have motivated us to look at this question. We show here that use of ^{41}Ca as a dating tool may indeed be feasible.

Most proposed cosmogenic dating nuclides are formed by the interaction of cosmic-ray primary and secondary particles with the Earth's atmosphere. The only significant components of the atmosphere which can give rise to ^{41}Ca are krypton and, with low energy α particles, argon. As the abundance of Kr in the atmosphere is only $\sim 10^{-4}\%$, and the effect of α particles small, production by this means is also small. Calcium is, however, a fairly abundant element in the Earth's crust. Here, the reaction ^{40}Ca (n, γ) ^{41}Ca can take place with slow neutrons. At the Earth's surface such a process is favoured both because of a fairly large cross-section ($\sigma = 440$ mb for thermal neutrons), and because the downward moving neutrons in the atmosphere are rapidly thermalised by the ground, giving rise to a significantly enhanced slow neutron flux at the air–surface interface[3].

The concentration of ^{41}Ca after exposure to a flux (f) of neutrons for a time (t) is given by

$$^{41}\mathrm{Ca}/^{40}\mathrm{Ca} = \frac{f\sigma t_{1/2}}{0.693}(1-\exp(-0.693t/t_{1/2}))$$

Using a value of 3×10^{-3} n cm^{-2} s^{-1} for the thermal flux[3], and $t \gg t_{1/2}$, we get a 'saturation' value of $^{41}\mathrm{Ca}/^{40}\mathrm{Ca} \sim 8\times10^{-15}$. This corresponds to an activity of $\sim1\times10^{-3}$ disintegrations per minute per gramme of Ca—about 10^4 times smaller than for contemporary ^{14}C. In addition, the decay of ^{41}Ca takes place by orbital electron capture directly to the ground state of ^{41}K. Therefore, the only radiation given off is the low energy (3.3 keV) X ray from the ^{41}K. It is these factors which have discouraged consideration of this isotope as a dating possibility.

Radioisotope detection with accelerators[4] has the advantage of high sensitivity, independent of the mode of decay. The technique has already been successfully applied[4–8] to measurement of ^{3}H, ^{14}C, ^{10}Be and ^{36}Cl, and shows great promise for some other long-lived isotopes (several variants of this technique are described in ref. 9). To measure a $^{41}\mathrm{Ca}/^{40}\mathrm{Ca}$ ratio of $\leqslant10^{-14}$, a machine capable of producing a ^{40}Ca beam of $\geqslant10^{13}$ particles per second would be desirable. As each gramme of Ca having the above ratio contains only $\sim10^{8}$ atoms of ^{41}Ca, we would also like to have an overall acceleration efficiency of $\geqslant10^{-5}$. For reasons given below, it might be necessary to accelerate the ^{41}Ca ions to an energy of $\geqslant$300 MeV. There is at least one accelerator in which Ca beams meet the above requirements[10]. Several superconducting or two-stage accelerators proposed or currently under construction should also have such a capability. Also, by including a 'pre-enrichment' step (for example, using an isotope separator), the above intensity requirement could be reduced significantly.

The main interference in such a measurement is expected to come from saturation of the detecting element with a large flux of natural ^{41}K. In this case the technique of absorbing the interfering ion[4] cannot be used because the range of the ^{41}K is greater than for ^{41}Ca. It may be possible to make a sufficiently good chemical separation of the sample to avoid this difficulty. However, an even more elegant technique would be to use the fact that ^{41}K can never have an atomic charge of +20. Thus, by accelerating the ^{41}Ca ions to sufficient energy ($\geqslant$300 MeV) for a significant fraction of them to be fully stripped, they can be magnetically separated from the ^{41}K. We have recently confirmed the feasibility of this technique by separating 200-MeV beams of ^{26}Al and ^{26}Mg at the ALICE accelerator at Orsay (unpublished work). Such a procedure would also be applicable to other isotopes, such as ^{36}Cl, ^{53}Mn and ^{59}Ni, which have nuclear charges greater than an interfering isobar.

To be of optimum use as a dating tool, the production and decay of the radionuclide must be clearly separated in time. With species formed in the atmosphere, this separation is quite natural. For a radionuclide formed in the lithosphere, such a separation means that the material being considered must be removed from (or exposed to) the source of the irradiation flux in a time which is short compared with the relevant half-life. For nuclear active bombarding particles such as neutrons, the shielding required to reduce production to negligible levels is of the order of 500 g cm^{-2}.

Some of the interesting possibilities of using isotopes formed in this way have been discussed by Davis and Schaeffer[11] in connection with ^{36}Cl. For example, one can determine when presently exposed samples were brought to the surface (such as by volcanoes, glaciers, or changing river patterns).

In the case of Ca, a potentially interesting application is the dating of $\mathrm{CaCO_3}$ in sediments. Here the 'age' measured would be the time since the ^{41}Ca was removed from its equilibrium concentration in the overlying water. The most promising environments for such an application are bodies of water (such as lakes) where the Ca residence time is short compared with the ^{41}Ca half-life. In the oceans, the estimated residence time is $\sim10^{6}$ yr (ref. 12). As this is about seven times the ^{41}Ca half-life, the equilibrium ratio of $^{41}\mathrm{Ca}/^{40}\mathrm{Ca}$ in the oceans is expected to be about two orders of magnitude less than the injection ratio. A $^{41}\mathrm{Ca}/^{40}\mathrm{Ca}$ ratio of 10^{-16} or smaller would almost certainly necessitate a pre-enrichment step before measurement with an accelerator. In addition, at such low concentrations one must begin to worry about other rare production modes, such as those induced by muon capture or natural radioactivity. However, even here a comparison of the injection ratio of $^{41}\mathrm{Ca}/^{40}\mathrm{Ca}$ with that found in various parts of the oceans (or sediments) might be useful for studying ocean circulation patterns.

The most exciting application of ^{41}Ca is that it might be used for dating animal (including hominid) remains in the interval of $\sim5\times10^{4}$–10^{6} yr: the bones of most vertebrates consist of calcium phosphate. Most of this Ca comes, directly or indirectly, from plants which have absorbed it from near surface water. The mean rate of erosion on the Earth's surface is estimated to be $\sim$5 cm per 10^{3} yr (ref. 13). Thus, on average, material will rest within 1 m of the surface for $\sim$20,000 yr. Of course, the actual values vary considerably, depending on the location, climate, type of rock and so on (for example, in low relief tropical regions, which may be among the most interesting for this application, the estimated surface residence time can approach 10^{6} yr (ref. 13)). In addition, being readily soluble, there can be exchange between surface Ca and that dissolved from deeper sources. At present we find it difficult to evaluate the overall effect of these factors. Ultimately they can probably only be determined by experiment. However, ignoring the effects of exchange, we estimate that surface water would typically have a $^{41}\mathrm{Ca}/^{40}\mathrm{Ca}$ ratio of $\sim10^{-15}$. In such a circumstance, ^{41}Ca would offer a possibility for dating in an analogous way to ^{14}C. One could take advantage of the fact that the living sites for early hominids were often grottos having the prerequisite shielding to stop production of ^{41}Ca. Thus the $^{41}\mathrm{Ca}/^{40}\mathrm{Ca}$ ratio in fossil remains in such sites, compared with the initial ratio, would be a measure of their age. Even if, as is likely, the initial $^{41}\mathrm{Ca}/^{40}\mathrm{Ca}$ ratio were not universally constant, it might be possible to 'calibrate' the technique at various epochs and locations, using dates obtained by other methods.

Although it will take a careful evaluation, and eventually experimental verification of the assumptions made here, we believe that radioactive dating using ^{41}Ca may turn out to be a very important procedure. We are optimistic enough to be currently working on an experimental test of these ideas.

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Strontium isotope evidence for petrogenesis of Central American andesites

THE active volcanic belts of Mexico and of Central America can be considered in two groups (Fig. 1). The Mexican volcanic belt (MVB) forms an irregular line trending away from the Middle America Trench, running from Ceboruco and Colima in the west to San Martin on the Caribbean coast[1]. In contrast to the MBV, the active Central American volcanic belt runs some 1,200 km from southern Mexico (Tacaná) and Guatemala to Costa Rica, broadly parallel with the Middle America Trench[1]. With the exception of the alkaline volcano San Martin[2], these are all calc-alkaline andesite volcanoes[1]. Between Mexico and El Salvador, the volcanic chain is built on a crust which includes Precambrian and Lower Palaeozoic crystalline rocks[3,4]. South-east of El Salvador, in Nicaragua and Costa Rica the crust is composed mainly of Cainozoic volcanic and sedimentary rocks resting on Cretaceous ultramafic complexes[3–6]. This change in basement character does not seem to be reflected in the composition of volcanic rocks[7]. Here we report new Sr isotope data for Cainozoic volcanic rocks from Costa Rica, and by comparing these with earlier published data we conclude that all of these volcanic rocks have their origin in the mantle and that a sialic contribution to their origin is insignificant.

Sr isotope data for Cainozoic volcanic rocks from Central America have been published for most of the area shown in Fig. 1. These include data for Mexico[8,9], Guatemala[10,11], El Salvador and Nicaragua[10], and Costa Rica[12]. In addition to the volcanic rocks, Pushkar has reported Sr isotope data for pre-Cainozoic basement rocks from Guatemala and Honduras[10]. For Costa Rica, the only reported Sr isotope data are seven analyses determined by Montigny *et al.*[12]. These include one and four samples respectively from the active volcanoes Irazú and Poás.

We have determined Sr isotope data for 10 Recent lavas and juvenile blocks from the active volcanoes Arenál (four), Poás (four) and Irazú (two). The lavas from Póas are Pleistocene–Recent, whereas those from Arenal were erupted during 1978, and the blocks from Irazú were erupted during 1963–64. Sr isotope ratios were determined on a VG-Micromass 30 mass spectrometer using chemical and mass spectrometric techniques described elsewhere[13]. Measurements of the Eimer and Amend standard SrCO$_3$ carried out during this work yield an overall mean of 0.70812 ± 1. Rb/Sr isotope ratios were determined by a precise X-ray fluorescence technique[14]. The Sr isotope ratios and related analytical data for the Costa Rican lavas are presented in Table 1. Because the analysed samples are all geologically young (<1 Myr, but most are <20 yr), the ^{87}Sr/^{86}Sr ratios presented in Table 1 are taken as initial ratios. These data are shown as a plot of ^{87}Sr/^{86}Sr against ^{87}Rb/^{86}Sr in Fig. 2, which also shows fields of some previously determined Sr isotope data.

Table 1 shows that Sr isotope compositions of Costa Rican lavas from Arenál and Poás show slight but significant internal variation within the range 0.7036–0.7042. The samples from Irazú have ^{87}Sr/^{86}Sr ratios of 0.7036–0.7037. Although there is considerable variation in Rb/Sr (similar to that shown by Mexican lavas) there is no correlation of ^{87}Sr/^{86}Sr with ^{87}Rb/^{86}Sr either for individual sets of data or for the data as a whole. The relatively imprecise Sr isotope ratios of 0.7037–0.7055 reported for Costa Rican lavas by Montigny *et al.*[12] are within the range of Sr isotope data we have reported for the Mexican andesite volcanoes of Nevado de Colima, Colima, and Ceboruco, and the alkali basalt volcano San Martin[9]. Sr isotope values (also determined in the Oxford Isotope Laboratory) for lavas from the Guatemalan volcanoes Santa Maria and Santiaguito are 0.70396 ± 6 (ref. 11) and hence indistinguishable from the values reported above. Earlier Sr isotope ratios determined for lavas from Guatemala, El Salvador and Nicaragua by Pushkar[10] fall within the range 0.7031–0.7049 (13 samples), but are mostly within the range 0.7037–0.7044 (11 samples; see Fig. 2). In contrast, Sr isotope values for pre-Cainozoic metamorphic and igneous rocks from Guatemala, Honduras and Nicaragua are largely within the range 0.71–0.75 (ref. 10).

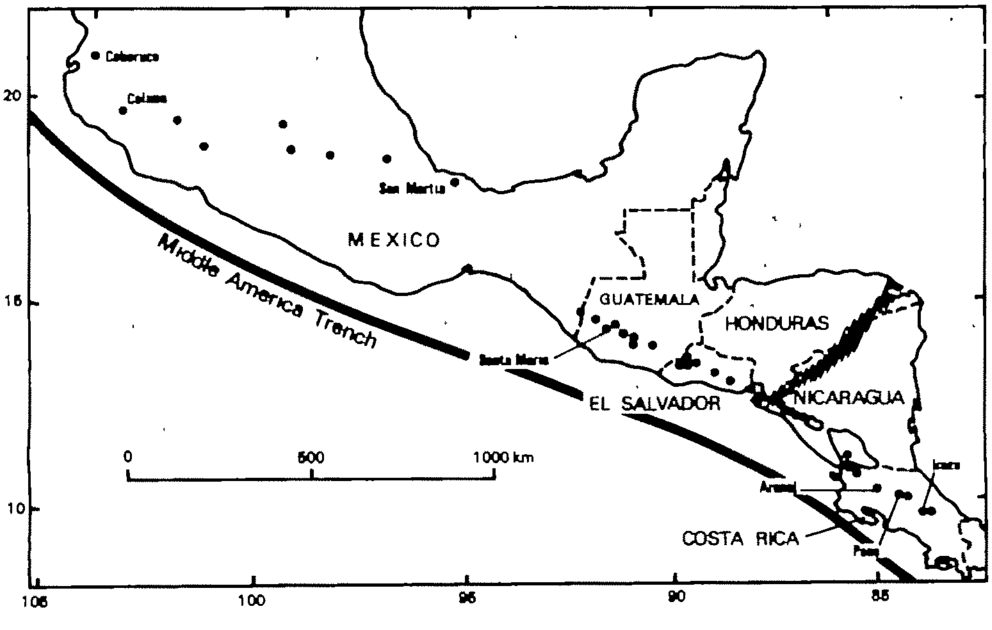

Fig. 1 Volcanoes and plate tectonics of Mexico and Central America. ●, Recently active volcanoes. The shaded area between Honduras and Nicaragua separates an area with older pre-Mesozoic crust to the north-west from an area with continental crust of upper Mesozoic–Cainozoic crust to the south-east. See text for further discussion.

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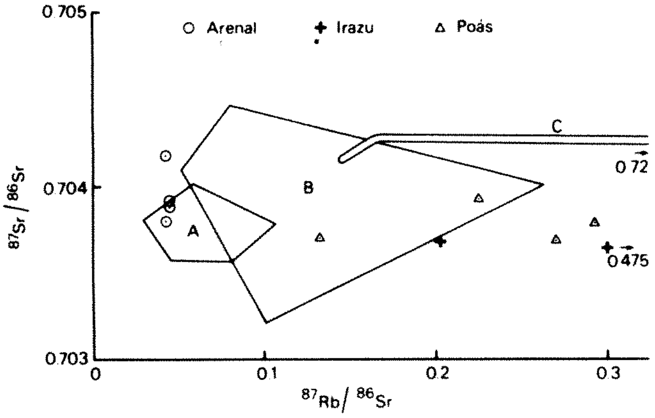

Fig. 2 Plot of $^{87}Sr/^{86}Sr$ against $^{87}Rb/^{86}Sr$ for volcanic rocks from Mexico and Costa Rica. The outlined fields A–C are for volcanic rocks from Mexico (refs 8 and 9). Field A is for lavas from San Martin, B is for lavas from the Colima and Toluca areas and C is for lavas from Ceboruco (see ref. 9, Fig. 2).

The above Sr isotope ratios for volcanic rocks of Mexico and Central America are all within the range reported for lavas from volcanic arcs where sialic crust is absent[10,15–17] and show no significant difference between the MVB and north-west Central America (pre-Cainozoic sialic crust) and south-east Central America (largly Cainozoic sialic crust). The low Sr isotope ratios for Mexican and Central American andesites, commonly within the range 0.7035–0.7040, suggest that sialic bulk contribution to their origin is insignificant.

The Sr isotope data provide clues to the origin and subsequent evolution of andesitic magma. First, the new results confirm that andesites from many oceanic and continental provinces are derived from a source region with $^{87}Sr/^{86}Sr$ ratios within a relatively narrow range of ~0.7035–0.7045 (see refs 9, 18, 19). This range is slightly but significantly higher than the source region of ocean floor basalts (0.702–0.703: ref. 18), and the data could be interpreted in terms of a model, developed from Sr and Nd isotope data, in which andesites are produced by partial melting of mantle wedge which has been enriched in ^{87}Sr as a result of fluids driven off the descending oceanic slab[24,25]. However, the Sr isotope compositions of the andesites lie within the range reported for oceanic and continental alkali basalts[9,18,20–23], and derivation from such a mantle source cannot be excluded[26]. Hence variation in the Sr isotope composition might result from small-scale mantle inhomogeneities. This cannot be evaluated without further Sr and Nd isotope studies, but data reviewed below suggest that some of the small-scale Sr isotope variations might result from processes occurring within the crust rather than the mantle.

The $^{238}U–^{230}Th$ radioactive disequilibrium method has been applied to Costa Rican lavas by Allègre and Condomines[27]. These workers analysed rock and mineral samples from Irazú (six samples, including a 'volcanic bomb' from the 1964 eruption, Poás (two samples) and Arenál (one sample). For the Irazú samples, $^{238}U–^{230}Th$ mineral isochrons indicate crystallisation of the lavas at times between 1.1×10^5 yr and the present day (equivalent to 1964). By considering the evolution of the initial $^{230}Th/^{232}Th$ ratio with time, Allègre and Condomines argue that the Irazú lavas evolved from a magma chamber established at 1.4×10^5 yr BP, with $^{230}Th/^{232}Th = 0.81$. Assuming a similar initial $^{230}Th/^{232}Th$ and source U/Th ratio, the samples from Arenál and Poás must have evolved from independent magmatic systems established respectively at 3.5×10^4 yr and 5×10^4 yr BP. These data may be interpreted as indicating the presence of a magma chamber below each of the three Costa Rican volcanoes. It seems plausible, therefore, that small variations in Sr isotope composition, both within and between volcanoes, are the result of processes (such as selective contamination) which take place within the magma chamber.

Table 1 Analyses of Costa Rican lavas

Specimen No.	Rb* (p.p.m.)	Sr* (p.p.m.)	$^{87}Rb/^{86}Sr$†	$^{87}Sr/^{86}Sr$‡
Arenál				
AR1	10.5	697	0.043	0.70417 ± 8
AR2	11.2	709	0.046	0.70392 ± 8
AR3	10.9	702	0.046	0.70389 ± 8
AR4	10.9	709	0.043	0.70380 ± 5
Poás				
P5	39.9	509	0.226	0.70392 ± 7
P10	48.7	486	0.289	0.70378 ± 7
P50	48.5	524	0.269	0.70365 ± 8
P52	26.5	580	0.133	0.70371 ± 7
Irazú				
IR1	54.4	780	0.203	0.70369 ± 7
IR2	105	643	0.475	0.70364 ± 6

* Determined by X-ray fluorescence. Mass absorption coefficients estimated from Compton scattering, concentration data about ±5%.

† Average precision ±1% (for X-ray fluorescence techniques, see ref. 14).

‡ Within-run precision quoted at 2σ error.

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Calibration of Grenvillian palaeopoles by $^{40}Ar/^{39}Ar$ dating

PALAEOPOLES from the exposed Grenville Province of the Canadian Shield are displaced from those of the remaining shield. It has been proposed that these poles record either a Precambrian plate collision ~1,000 Myr (refs 1–3) or manifest a large rotation and translation of the entire extant North American crust[4–6]. Geological evidence in support of the collisional hypothesis is largely lacking; for example, an acceptable suture zone has not been located. The critical evidence for a choice between the two-plate and one-plate tectonic hypotheses must, therefore, come from dating these palaeopoles because if the magnetisations were acquired contemporaneously with those of the Logan Loop (≈1,000–1,200 Myr), then the two-plate or collisional hypothesis is probably correct. We present here an empirically derived cooling curve for the Haliburton basic intrusions within the Grenville Province. These intrusions are located in a region of high-grade metamorphism, upper amphibolite to granulite facies. Their emplacement times are not known, but in the Haliburton Highlands cooling from the culmination of regional metamorphism, not contemporaneous throughout the Grenville Province, began ~1,050 Myr ago[7]. This is the first cooling curve for an orogen to be derived solely from isotopic data; that is, the first for which both temperature and time have been determined from the same samples. Using this as a calibration curve, we show here that the thermal remanent magnetisation (TRM) components from the Haliburton basic intrusions were acquired between ~980 and 820 Myr ago during slow cooling and that, consequently, the two-plate hypothesis is improbable.

With the rapid extension in recent years of palaeomagnetism to Precambrian rocks in orogenic belts and the subsequent discovery of multicomponent natural remanent magnetisations (NRMs) (see ref. 8 for references), it has become clear that the acquisition times for TRM components are not always directly defined by an isotopic date. In slow cooling conditions, the Rb–Sr whole-rock and K–Ar mineral clocks will record different times. Hence, the TRM acquisition times will be indeterminate. Furthermore, for a history of successive reheating episodes, rather than one of slow cooling, the conventional K–Ar and Rb–Sr dating techniques often lack the requisite discrimination so that partially reset dates will be confused with totally reset dates.

To overcome these limitations, we have applied detailed (15-step) incremental heating, $^{40}Ar/^{39}Ar$ experiments to a variety of minerals, including some types traditionally shunned in K–Ar dating. These minerals were chosen from the same samples studied palaeomagnetically[3] from the Bark Lake and Dudmon dioritic bodies and the Glamorgan gabbro-anorthosite complex in southern Ontario. Using the $^{40}Ar/^{39}Ar$ method as a diffusion experiment, we have calculated Ar closure temperatures for each mineral with Dodson's formula[9], as first suggested by Buchan *et al.*[8]. The required diffusion coefficients were calculated by the method of Fechtig and Kalbitzer[10]. These closure temperatures are insensitive to uncertainties in the assumed cooling rate[9] and so the formula can most profitably be applied to Precambrian orogenic belts where a slow cooling following uplift and erosion is likely to have occurred. By thus establishing a cooling curve from isotopic studies, we can directly date TRMs using their empirical blocking temperatures.

In detailed palaeomagnetic studies[3,8], the Haliburton intrusions have yielded three NRM components, two of which are probably partial TRMs (PTRM) and sometimes occur within the same specimen. The component with the highest laboratory blocking temperature gives a palaeopole, Hb_1, at the southern limit of the Grenvillian poles and near southeastern Australia, and the second PTRM component has a pole, Hb_3, at the northern limit (Fig. 2). The inferred direction of apparent polar wander (APW) is from south to north[1–3]. As a reasonable approximation in this first attempt at the direct dating of multicomponent TRMs from orogenic belts, we used field blocking temperatures estimated by the conventional static temperature method[8,11,12] rather than the kinetic temperature approach developed by York[13,14] that is formally analogous to the approach used here with isotopic closure temperatures. From the theoretical curves of Pullaiah and others[11], we estimate that the bulk of the Hb_1 PTRM component carried by magnetite was acquired in the range 520–570 °C and that the bulk of the Hb_1 component carried by hematite, if PTRM, was acquired in the range 600–650 °C. We thus assign a probable maximum acquisition range for the Hb_1 component of 520–650 °C by the static method. Similarly, field temperatures of not more than 250 °C could explain the laboratory blocking temperatures up to 450 °C for the Hb_3 PTRM carried by a titanomagnetite. We thus have an ideal situation for dating, having rocks with PTRMs acquired ~300 °C apart and with palaeopoles about 60° apart latitudinally straddling the entire group of Grenvillian palaeopoles.

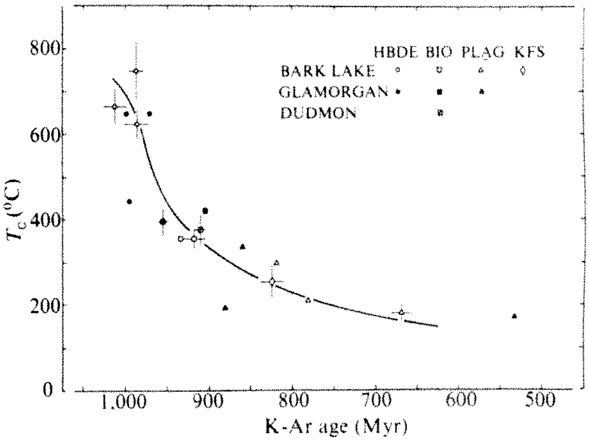

Fig. 1 An isotopically derived cooling curve for the Haliburton area using revised decay constants[16]. A cooling rate of 5° Myr^{-1} was assumed in Dodson's formula for all but the three rightmost points, for which 0.5° Myr^{-1} was chosen. The error bars are 1σ.

We have dated six hornblendes, five relatively coarse biotites, six plagioclases and one potassium feldspar. Experimental details will be described elsewhere. All of the biotites and four of the hornblendes as well as the K-feldspar gave excellent plateaus over at least 90% of the released Ar. There were also significant age differences among the biotites, even from the same intrusions, and between the biotites and hornblendes. These remarkable facts can best be interpreted as due to a slow-cooling history and are least compatible with a history comprised of a sequence of episodic thermal events. Such events cannot partially reset coarse biotites and the sensitive K-feldspar without leaving anomalous and irregular age spectra[15]. One of the interesting discoveries in this project was that plutonic plagioclases, previously considered unreliable K–Ar clocks, yield plateau segments in the laboratory temperature range of ≈700–900 °C and that these plateaus have chronological significance. Specifically, in the only sample with coexisting K-feldspar and plagioclase, the plateaus for these minerals are concordant. In calculating the diffusion coefficients for plagioclases, we have treated the Ar corresponding to the plateau segments as an independent phase. We used two conservative criteria to classify Ar closure temperatures as reliable or unreliable. First, a plateau consisting of at least five consecutive gas fractions must exist. Second, the diffusion coefficients corresponding to at least these five steps must lie on a statistically well defined straight line in an Arrhenius diagram.

The overall pattern of closure temperatures shown in Fig. 1 is internally consistent with a slow cooling interpretation and the

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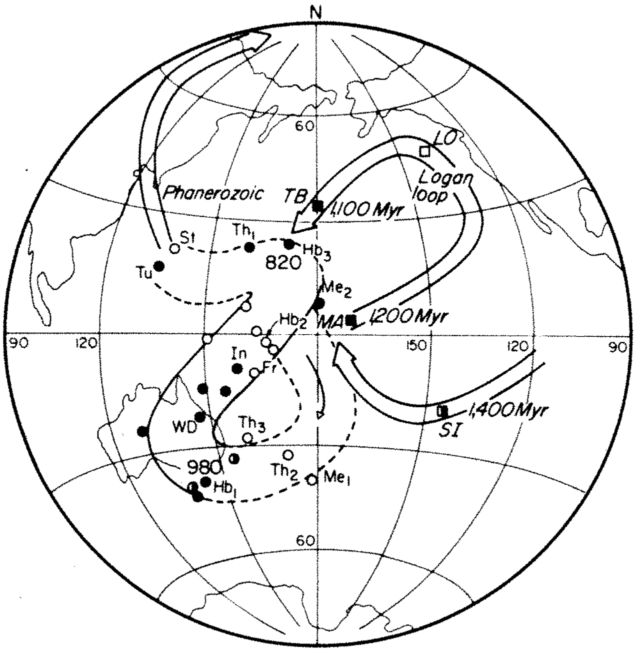

Fig. 2 A proposed Grenville Loop APW path. The calibrated part (solid) corresponds to a minimum average drift rate of 5 cm yr^{-1}. The symbols and identity of unlabelled poles are given elsewhere[3,17].

temperatures are geologically reasonable. For example, those for the hornblendes are less than the expected maximum temperatures ($\simeq$700 °C) during the peak of metamorphism in this area[18]. Therefore, with this cooling curve, we assigned acquisition times of 980 ± 10 Myr (520–650 °C) for component Hb_1 and $\lesssim$820 Myr ($\lesssim$250 °C) for Hb_3. In this way, we have calibrated the APW path for many of the Grenvillian palaeopoles.

All of the known reliable Grenvillian palaeopoles are plotted in Fig. 2 as well as poles for the Tudor gabbro (Tu) and Steel Mountain anorthosite (St) which occur within the Grenville Province. Superimposed is our proposed Grenville Loop with the calibrated track bounded by solid lines.

Although the shape, position and sense of direction of this loop have already been proposed[19,20], the new time limits significantly alter the previous tectonic interpretations. Our calibrated track represents a minimum average drift rate of $\simeq$5 cm yr^{-1}, certainly more reasonable than the rates of 10–20 cm yr^{-1} deduced from some previous tectonic interpretations[19–21]. Earlier proposed Grenville Loops that lap back on themselves[21,22] have used very approximate and indirect age assignments and should be viewed as first approximations to more refined definitions of APW paths based on direct dating procedures such as that outlined here. We must reject the two-plate hypothesis because the ages assigned to our calibrated track are much younger than required by that hypothesis ($\simeq$1,000 Myr north of the Equator on the northwards segment).

There is support for this calibration from other dating studies. Reynolds[23] has assigned a $\simeq$910 Myr date to the Frontenac Axis dikes (Fr) pole, previously thought to be $\simeq$690 Myr. Dallmeyer[24] has placed the Indian Head (In) pole at $\simeq$900 Myr. Dallmeyer and Sutter[25] assigned a date of 980–1,010 Myr to the Whitestone diorite (WD) pole. Although the confidence ovals for the Hb_1 and WD poles overlap slightly, by interpolation in Fig. 2, the WD magnetisation has probably been acquired in the 900–980 Myr interval. The main cause of the apparent discrepancy between this acquisition time and that suggested by Dallmeyer and Sutter is their choice of a much lower closure temperature (500–550 °C) for their hornblende (see ref. 26). We think such estimates from the literature are only first approximations (they ignore sample differences, for example) and may yield only lower limits to this temperature.

The dashed, southwards track is uncalibrated but does contain poles of probable age 1,000–1,100 Myr such as the Cardenas Lavas and Pikes Peak Granite poles[21] those from the Jacobsville sedimentary formation[20] as well as those of the Nonsuch shale and the Freda sandstone[27]. Pole Me_1 from the Mealy Mountain anorthosite[4] and poles Th_3 and Th_2 from the Thanet gabbro in the Hastings Basin[17], if TRM components, are predicted from Fig. 2 to have acquisition times of $\simeq$980–1,020 Myr. The dashed segment between Hb_3 and Tu is entirely speculative because no reliable date can be assigned to Tu or St. Although the palaeomagnetic literature often quotes a 680 Myr date[28] for Tu, the original authors[28] emphasised their uncertainty concerning the event represented by this date. A further complication is the presence of North American Cambrian poles within a swath roughly from Tu to Fr[21,29]. We are therefore unsure how an APW path should be constructed after Hb_3.

We reject the Hadrynian Track of Morris and Roy[19] and the Hadrynian Loop of Irving and McGlynn[21] because not only are the dates of some of their critical rock units very suspect (Tudor gabbro, Franklin diabase and Aillik dikes) or incorrect (Frontenac Axis dikes), but also the time of acquisition of the Stoer Group magnetisation may be quite different from the precise 970 Myr (new decay constant) Rb–Sr date. Rb–Sr dates on sedimentary rocks are ambiguous[30,31].

In conclusion, through the exploitation of the $^{40}Ar/^{39}Ar$ incremental heating technique as both a diffusion experiment and a method of dating, we have calculated a cooling curve for the Haliburton intrusions and thereby have calibrated the northward track of a proposed Grenville Loop. The assigned dates are incompatible with the collisional or two-plate hypothesis as an explanation of the displaced Grenvillian palaeopoles. This is not to say that such a collision did not occur or did not influence the so-called Grenville Orogeny but rather that it has not been recorded palaeomagnetically. What is recorded is a translation and rotation of Grenvillia and Interior Laurentia[21]. The role of any such collisional process is likely to have been similar to that proposed by Dewey and Burke[32] with a collisional zone south-east of the Grenville Province and now hidden under the Appalachians.

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Pore water sampling in anoxic carbonate sediments: oxidation artefacts

ANY exposure of anoxic estuarine sediments to the atmosphere during sample handling and processing can lead to decreased values of ferrous iron[1], reactive phosphate[2] and reactive silicate[3] in the pore waters of these sediments. Thus, the mishandling of anaerobic sediments and their pore fluids will in turn lower calculated diffusional fluxes of these compounds from the sediments into the overlying water. To date, this oxidation effect has only been documented for clastic sediments rich in iron. We present data here which indicate that a similar phenomenon occurs when anoxic iron-poor, carbonate-rich sediments are exposed to an oxidising milieu.

A core was taken during June 1978 from a shallow water sub-tidal area in Coot Bay on the northeastern shore of Bermuda. Previous data[4,5] collected in the summer of 1977 indicated that the sediments were anoxic and alkalinity and nutrient regeneration was occurring. The core was obtained by hand by carefully pushing a 6.6 cm i.d. polycarbonate core liner into the sediment.

On retrieval, the core was placed into a polyvinyl chloride core carrier, purged with nitrogen and returned to the Bermuda Biological Station for Research. The time between the taking of the sediment sample and the initiation of the pore water extraction in a nitrogen-filled glove bag was ~15 min.

The core was extruded in the glove bag under nitrogen and sectioned into six 10-cm segments. While in the glove bag, the core sections were cut in half vertically using a Teflon coated spatula. One half of the six core sections was placed into a 500 ml linear polyethylene (LPE) centrifuge bottle which had been previously cleaned for trace metal analysis[6]. The bottles were centrifuged at 10,600*g* at exactly *in situ* surface sediment temperatures under nitrogen.

This set of centrifuged samples was placed back in the nitrogen-purged glove bag and filtered through precleaned 0.4-μm Nuclepore filters[3]. The filtered samples (10 ml) were then pipetted into a 60-ml conventional polyethylene (CPE) bottle and within 30 min were analysed for *p*H and titration alkalinity. The titrated sample was later analysed for sulphate, calcium, phosphate and reactive silicate. The remainder of the filtered sample was decanted into a 60-ml CPE bottle which had been cleaned as described by Patterson and Settle[6] and immediately acidified to 1% with concentrated Ultrex nitric acid for later trace metal analysis.

The other set of samples was processed in a similar manner except for two important differences: (1) the samples were removed from the N_2 atmosphere and exposed to the atmosphere of the laboratory for 10 min before being placed in the centrifuge bottles; (2) the filtration and pipetting steps were carried out in the laboratory atmosphere instead of in the glove bag. The filtering and pipetting of the supernatant pore water took ~5 min for each sample and while one sample was being processed the others were kept in the glove bag under nitrogen. Therefore the maximum atmospheric exposure for each sample was 75 min.

Phosphate analyses were run on a Technicon Auto Analyzer II system[7] after a 2 : 1 dilution. The differences between duplicate dilutions never exceeded 2%. Reactive silicate was analysed using the method of Strickland and Parsons[8] modified for pore water by Presley[9]. Replicate analyses indicate a standard deviation of $\pm 1\ \mu\mathrm{M\ l^{-1}}$. Total reactive iron was analysed colorimetrically[10] using the Technicon Auto Analyzer II system[11] with a coefficient of variation of $< \pm 0.05\ \mu\mathrm{M\ l^{-1}}$. The samples were never frozen and all the analyses were carried out within 5 weeks of sample collection.

We found that there was a decrease of Fe of 81% in the top 25 cm of the core when the samples were exposed to laboratory air compared with the subsamples processed under nitrogen (Fig. 1). These two sections have iron concentrations $>1.80\ \mu\mathrm{M}$ ($100\ \mu\mathrm{g\ l^{-1}}$). Below this concentration the dissolved iron is apparently not affected by the atmospheric exposure. The dissolved phosphate data parallel the iron in that the greatest differences between the nitrogen processed samples and those exposed to the laboratory atmosphere are in the two uppermost core sections (Fig. 1). There is also a significant decrease in the PO_4^{3-} concentration in the lowest core section where the iron concentration is little affected. The reactive silicate values, with the exception of the 35-cm depth sample, are all lower in the laboratory exposed samples. There were no significant differences in the concentrations of *p*H, SO_4^{2-}, Ca^{2+}, Mn^{2+} and titration alkalinity between the nitrogen processed and the laboratory exposed subsamples.

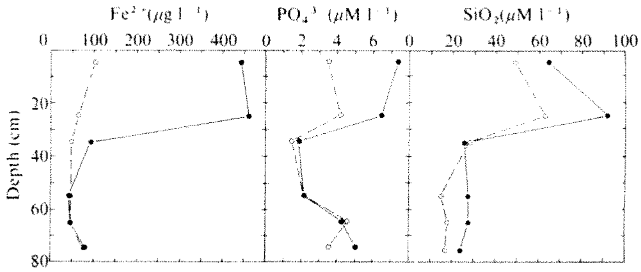

Fig. 1 Pore water concentrations of ferrous iron (Fe^{2+}), phosphate (PO_4^{3-}) and silicate (SiO_2) for a core from Coot Bay, Bermuda. ○, Samples processed after atmospheric exposure; ●, samples processed under N_2.

It seems that there is a critical dissolved iron value where laboratory exposures of as long as 75 min has no effect on the total dissolved iron concentration in the pore waters. Below this critical iron concentration, phosphate is also little affected by the presence of oxygen. The oxidation of Fe^{2+} to Fe^{3+} is very rapid at neutral *p*H (refs 12, 13). Whether this dissolved iron is in a physicochemical state that is unaffected by the increased partial pressure of oxygen is unknown. The iron may be associated with dissolved organic matter[14] (DOM) and the kinetics of its oxidation may be much slower than that of ionic Fe (ref. 15). It is interesting that pore water iron data collected before the knowledge of oxidation effects tend to show 'residual' dissolved iron values close to $100\ \mu\mathrm{g\ l^{-1}}$ (refs 16, 17).

Reactive silicate, however, is continually lost by laboratory atmospheric exposure even when the dissolved iron is apparently not being oxidised. These data indicate that the mechanism of silicate removal may not be directly related to the oxidation of Fe^{2+}. It is not known what governs the loss of silicate during oxidative handling and processing, nor whether the removal of SiO_2 without the oxidation of Fe^{2+} occurs only in calcareous sediment.

The data from the top 25 cm of core indicate that for every $10\ \mu\mathrm{M}$ of iron removed during oxidation $4.5\ \mu\mathrm{M}$ of phosphate is lost. More phosphate per mol of iron is lost in these calcareous sediments than from the anoxic clastic sediments studied by Loder *et al.*[3]. This may be related to the exposure time of the pore water sample to the oxygen atmosphere rather than the effective scavenging potential of the iron hydroxide formed. The iron-rich clastic sediments of Loder *et al.*[3] were only exposed for a period of 12 min. The Coot Bay data indicate

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that an average of 28% of the reactive silicate is lost during the conditions of the experiment. This is double that found by Loder *et al.*[3] for clastic sediments.

Our experiments and those of Bray *et al.*[2], Troup *et al.*[1] and Loder *et al.*[3] emphasise the importance of proper sample handling if meaningful nutrient data from anoxic pore waters are to be obtained. Our data indicate that decreases of phosphate and reactive silicate in pore waters from anoxic carbonate-rich sediments do occur unless proper care is taken to ensure the lack of atmospheric exposure. Because pore water data are of extreme importance in understanding diagenetic pathways in marine sediments,[18,19], and pore water exchange with overlying water may be extremely important in the fluxes of nutrients and trace metals,[20-22], the collection of pore waters from reducing sediments must be accomplished with great care, if valid geochemical models are to be produced.

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A pre-2,000 Myr old granulite facies metamorphosed evaporite from Caraiba, Brazil?

THE identification of the first occurrence on Earth of sediments of evaporitic origin formed from seawater is obviously important not only for the study of the chemical evolution of ocean water but also in understanding the development of common sedimentary rock assemblages and the stabilisation of cratons. Until recently[1,2] confirmed pre-2,000 Myr old evaporites were unknown and indeed Ronov[3] maintained that they did not exist. Pyroxene granulite facies metamorphosed evaporites are also extremely rare[1,4] and thus a pre-2,000 Myr old example is important. We give here a brief account of the mineralogy of some of the rocks found in the Caraiba mine area of Brazil which suggests that evaporites existed earlier than previously recognised and were associated with dolomitic rocks.

The Caraiba copper deposit of Brazil is in the north of the state of Bahia, 9°52′ S, 39°52′ W (Fig. 1) being rich in chalcopyrite and bornite and is associated with a sequence of high grade gneisses, pyroxene granulites, migmatites and possible meta-evaporites which together form the Caraiba supergroup, set in the eastern margin of the Sao Francisco craton. Granite gneisses which cut these high grade rocks are about 2,050 ± 70 Myr by Rb–Sr age determination, corresponding to the Transamazonian orogeny, so that the earlier rocks are older than this date and some charnockitic types in the Caraiba area have Rb–Sr ages of 2,900 Myr so that the metasediments could well be Archaean.

The predominant rock type in the Caraiba mine area is enderbitic charnockite with biotite and this encloses the calc silicate proposed meta-evaporites but other rocks, such as granite gneisses, basic and ultrabasic granulites and pegmatoids come into contact with the possible meta-evaporites which are predominantly calc silicate diopsidites. Subordinate diopside–forsterite marbles, anhydrite–diopside ± K feldspar granulites also occur. Dolomite has not been identified in the carbonate bearing rocks. Only the diopsidites outcrop, the anhydrite-bearing rocks being obtained from drill-cores. Scapolite is present in some samples. The principal host rock for the chalcopyrite–bornite mineralisation is hypersthenite, and the mineralisation is thought to be magmatic, but the calc silicate rocks in contact with mineralised ultramafic or mafic rocks usually have some mineralisation. The occurrence of xenoliths of diopsidites in the hypersthenite confirms the older age of the diopsidites.

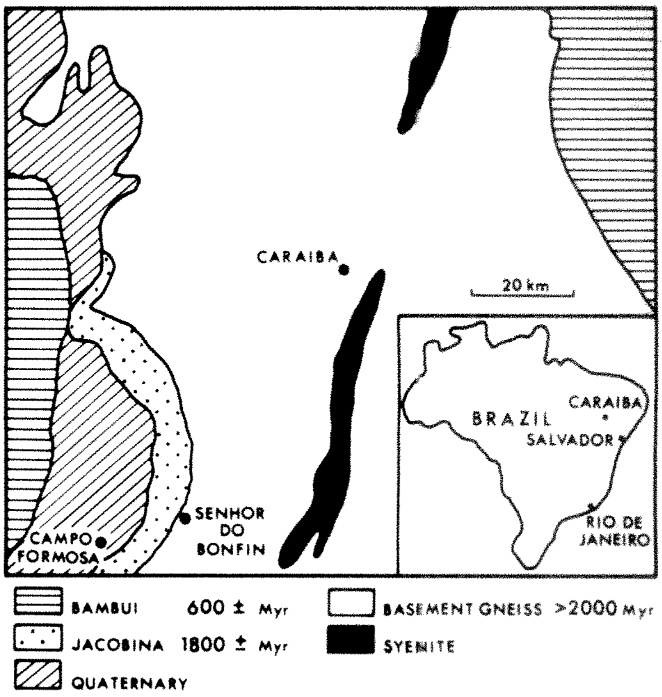

Fig. 1 Geological sketch map of the Caraiba area.

Samples range from the principal assemblage which is pure diopsidite, sometimes with green spinel, accessory apatite and sphene through dominantly two phase diopside—anhydrite rocks with variable amounts of phlogopitic mica, olivine (forsterite), microcline, sphene and grossular garnet to samples rich in calcite. Sometimes diopsidite grades into plagioclase and sphene bearing rocks.

A coarse (3–5 mm) granular specimen with 51.6% diopside, 47.5% anhydrite and 0.9% phlogopitic mica contains nearly colourless diopside ($2V_\gamma$ 55½; $Z \wedge \gamma$ 41 ± 1°) with an unzoned composition of 54.10% SiO_2, 1.79% Al_2O_3, 16.74% MgO, 24.18% CaO and 2.07% FeO (all Fe calculated as FeO), total 99.51% determined by electron microprobe analysis using energy dispersion. This is a composition close to pure diopside with $Ca_{0.97}(Mg_{0.91}Fe_{0.06}Al_{0.05})_{1.02}Si_{1.97}Al_{0.03}O_6$, and contains

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the low Al typical of many metamorphosed calc silicate rocks. The anhydrite (2V_γ 42°) has excellent cleavages and repeated lamellar twinning, and does not contain more than a trace of Sr. Such a rock, with only about 1% Al_2O_3 was clearly originally almost free of any clay or aluminosilicate minerals and was probably largely gypsum or anhydrite with diagenetic or detrital silica and perhaps a trace of illite. At one horizon olivine–anhydrite diopside samples occur with Fo_{94}.

Other samples are finer grained and contain grey, non-perthitic microcline which probe analysis shows to be nearly pure $KAlSi_3O_8$, accessory sphene containing ~1.5% Al_2O_3, accessory pyrite and diopside with similar low Al_2O_3 (1.73%) to the above but with more TiO_2 (0.35%) and FeO (5.20%). The extreme end-member contains no anhydrite and is a fassaite–calcite–phlogopitic mica (mol Fe/(Fe+Mg) = 0.21) assemblage that clearly contained much original aluminosilicate, presumably at least in part illite. the pyroxene in such samples varies in composition but 49.4% SiO_2, 5.8% Al_2O_3, 0.4% TiO_2, 13.2% MgO, 5.1% FeO (total), 0.5% MnO, 24.4% CaO is typical, giving $Ca_{0.98}(Mg_{0.74}Fe_{0.16}Mn_{0.01}Ti_{0.01}Al_{0.11})_{1.03}Si_{1.85}Al_{0.15}O_6$. This clearly reflects both the higher Al content of this rock, which is demonstrated by the quantity of mica (10%) and its Al_2O_3 content (16%), and the high grade of metamorphism that facilitated the incorporation of substantial Al in the pyroxene. This assemblage clearly arises from an impure siliceous or silicified dolomite. In view of the predominance of diopsidite the commonest original sediment was presumably dolomite.

Other samples near the calcite–pyroxene rich assemblages contain a highly altered unidentified sheet-like mineral containing about 38% SiO_2, 20% Al_2O_3, 24% CaO with 4% MgO + FeO, and now partly replaced by calcite and small (0.04 mm diameter) euhedral nearly pure grossular garnet of average composition 40.02% SiO_2, 22.14% Al_2O_3, 37.57% CaO, 0.75% MnO and less than 0.20% FeO and MgO with a slightly MnO enriched margin (0.91%) compared with the core (0.57%). Other grains of this highly altered mineral are replaced by clinozoisite.

These preliminary results, obtained with an electron microprobe, are under further investigation, but they may support the recent indications[1,4] that evaporites existed earlier than previously generally recognised[3] and were associated with dolomitic rocks, a common association in most younger evaporite deposits. However, it is possible that the anhydrite could have formed from dolomite by SO_2 attack, the SO_2 being derived from oxidation of chalcopyrite to bornite and magnetite during the metamorphism, for some magnetite is ubiquitous. Post metamorphic oxidation is notably lacking and fresh sulphides occur in outcrops. Accordingly, the evaporitic origin is not certain.

Any original halite would obviously have migrated from such a high grade metamorphic position but if the deposit was originally a sabhka-type evaporite there may not have been very much halite. The olivine, spinel and calcite would be derived from dolomite breakdown in well-known reactions.

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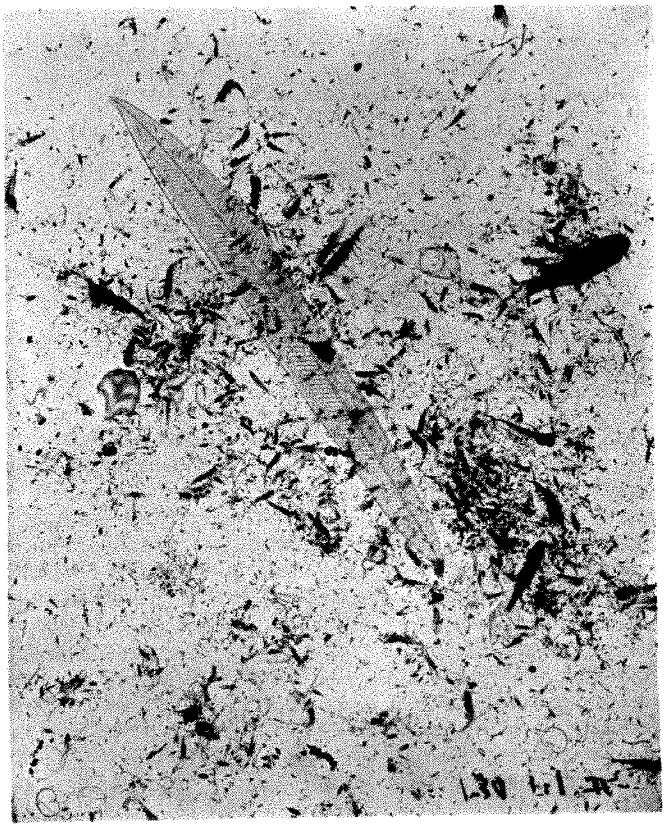
Silhouette photography of oceanic zooplankton

NO adequate method has been available to characterise a sample of zooplankton quickly and accurately. As Mullen noted[1], although relatively rapid, measures of standing crop such as displacement volume, dry weight, carbon content and electronic size spectra do not distinguish clearly between animals and plant or detrital material, much less between herbivores and carnivores. Direct microscopic enumeration has remained the only approach giving information detailed enough for many ecological studies. Unfortunately, this method is time-consuming, it cannot be undertaken routinely at sea, and it is feasible only after chemical agents have been added to 'fix' and then 'preserve' the sample. With time, plankton samples always deteriorate in preservatives, although the degree of this effect varies with species. We report here that a little-known photographic technique—high-speed silhouette photography[2,3]—can be used to analyse live zooplankton samples at sea.

The photographic system we used consisted of 8 × 10 inch Eastman fine-grain positive film 7302, an electronic flash with a xenon bulb (E.G. & G., Inc., FX6A) and a pulse duration of 3×10^{-6} s, and a plastic tray. Lamp-to-film distance was ~1.0 m. Immediately after collection, whole samples or aliquots of particularly dense samples were poured to a depth of about 1 cm into the plastic tray directly on top of the film. A glass plate was used to seal the top of the tray when seas were rough. One flash in a darkened room exposed the film. Samples were then preserved in the usual fashion (8% buffered formalin, pH ~8.2). Because of the shortness of the flash, action was stopped and results were unaffected by the motion of the ship or the subjects. Essentially any container and any powerful electronic flash could be used. The latter should be arranged to approximate a point-source as closely as possible. We housed the small-diameter xenon bulb in a non-reflective tube.

Fig. 1 Actual-size silhouette of a live zooplankton sample collected in the Sargasso Sea.



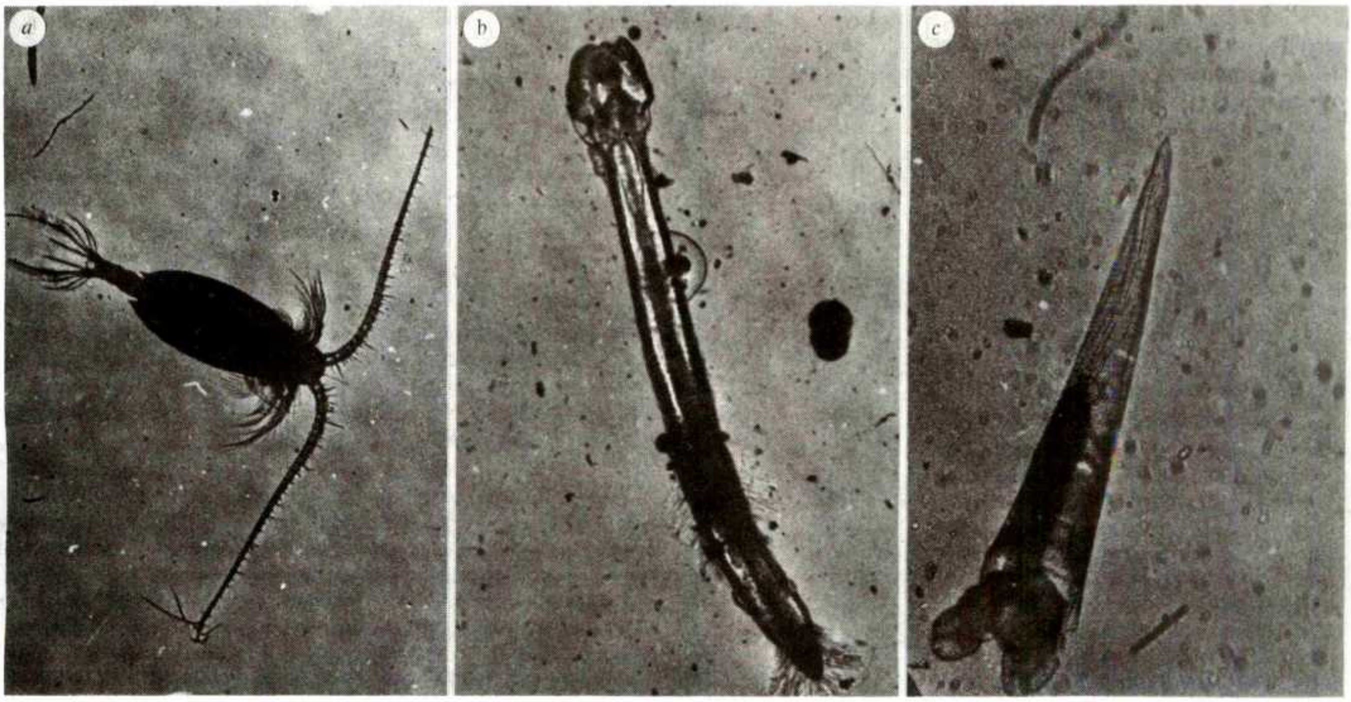


Fig. 2*a* Enlarged silhouette of a copepod, *Undinula vulgaris* female, ~2.5 mm. *b*, Enlarged silhouette of a chaetognath, *Pterosagitta draco* ~8 m. A fish egg lies just beyond the body trunk. *c*, Enlarged silhouette of a shelled pteropod, *Styliola subula*, ~2 mm.

Figure 1 is the contact print of a negative produced as described above. The sample was collected between 0 and 89 m depth in the southern Sargasso Sea in February 1978 and represents 443 m^3 of seawater filtered through the 0.333-mm mesh of a MOCNESS net system[4]. The relatively large leaf-like animal is an eel larva at the leptocephalus stage.

Such negatives were generally examined at sea by placing them between two glass plates and counting random transects but, also, we have projected them on a viewing screen. To be enumerated in a negative, the density of organisms first would be considerably reduced by subsampling. Because the area of a negative corresponds to a known volume of ocean, it can be used to characterise quantitatively the plankton of a region if resolution were sufficient.

The resolution obtained by our optic-less imaging technique was more than adequate for this purpose. It was possible to make generic and, occasionally, specific identifications of specimens in many cases. Figure 2*a–c* shows enlargements of individual animals from actual-size negatives such as Fig. 1. The animals were alive when they were photographed. The copepod in Fig. 2*a* was moving rapidly and erratically. The chaetognath in Fig. 2*b* is easily identified even though the collarette is nearly gone, as frequently occurs in net collections. The pteropod mollusc in Fig. 2*c* has its wing-like flaps partly extended in their swimming position. The tissue of a preserved specimen typically would have contracted into an indistinguishable lump. A negative such as Fig. 1 could not have been obtained by conventional photographic or photomicrographic techniques, which do not give the breadth of the field of view and the depth of focus required to accommodate the entire sample, nor the high resolution we obtained despite ship and animal motion.

The rapidity, ease, and low cost of silhouette photography of plankton make it potentially useful to field workers with limited facilities. It can produce a permanent record of a live sample, which is unobtainable at present by any other means. Most important, the technique is non-destructive. Preservatives can be added after the image is recorded, and the sample can be sorted and examined on shore.

An *in situ* silhouette camera system designed to assess zooplankton patchiness has already been tested and a programme has been initiated to fully automate enumeration of our negatives by means of computer image-analysis techniques.

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Drinking behaviour induced by intracerebroventricular administration of enkephalins to rats

INTRACRANIAL administration of substance P to rats inhibits drinking induced by water deprivation, sodium-chloride load, and intracerebroventricular (i.c.) injection of angiotensin II or carbachol[1]. It has been suggested that substance P is a neurotransmitter used in primary afferent fibres terminating in the spinal cord[2–4], but recent findings suggest also that, at least in mice, substance P may release endogenous opioids and thus produce analgesia[5]. If cerebral effects of substance P are, at least in part, due to the release of endogenous opioids, these substances might also possess an antidipsogenic effect. We describe here experiments in which we observed that enkephalins administered into the lateral ventricles of rats inhibited drinking induced by water deprivation or by i.c. injection of angiotensin II. We thus provide evidence that enkephalins have a potent and reproducible antidipsogenic effect in the rat.

In a preliminary operation, i.c. cannulae (o.d. 0.6 mm) were implanted stereotaxically in male Wistar rats. The animals weighed 250–300 g and were under Equithesin anaesthesia (3 ml per kg). They were allowed at least 10 d before testing began. Injections were made in conscious rats through a stainless-steel injector (o.d. 0.3 mm) temporarily inserted into the guide cannula. The drugs were dissolved in Tyrode solution and injected in volumes of 1 μl. Drinking was induced by a 16-h period of water deprivation or i.c. injection of angiotensin II, 100 ng per rat. The drugs used were angiotensin II (AII, synthetic 5-isoleucine-angiotensin II; Calbiochem), (Leu^5)-enkephalin (LEN), (Met^5)-enkephalin (MEN) and (D-Ala^2, D-Leu^5)-enkephalin (AEN) (all Wellcome).

In AII-treated rats, LEN and MEN inhibited drinking, but the effect required large amounts of peptide (100–200 μg per rat). Drinking inhibition was apparently dose dependent (Fig. 1*a*, *b*) and, as indicated in Fig. 1*d*, when latency between drug injection and the beginning of water intake was recorded, a clear-cut inhibition was also seen at relatively low doses (25–50 μg per rat).

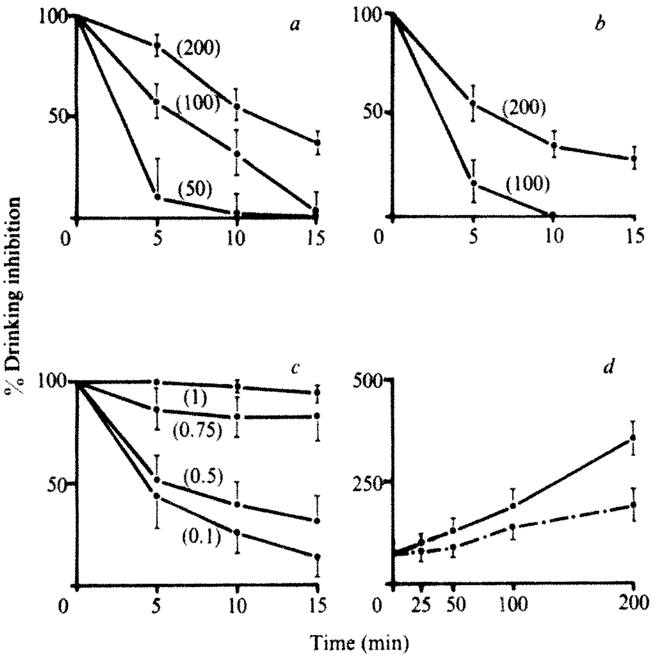

Fig. 1 Angiotensin II-treated rats. Inhibitory effect of (Met^5)-enkephalin (*a*), (Leu^5)-enkephalin (*b*) or (D-Ala^2, D-Leu^5)-enkephalin (*c*) on water intake induced by i.c. injection of angiotensin II, 100 ng per rat. In parentheses, doses in μg. Latency time after i.c. administration of (Leu^5)-enkephalin (continuous lines) or (Met^5)-enkephalin (dotted lines) is shown in *d*. Points and vertical bars represent means ± s.e. of 6–8 experiments.

AEN was about 2,000–4,000 times as active as the naturally occurring enkephalins LEN and MEN. In fact, a complete inhibition was attained with 1 μg of AEN (Fig. 1*c*), whereas 0.5 μg of the peptide had almost the same inhibitory effect as 100 μg LEN and 200 μg MEN.

In water-deprived rats, LEN and MEN inhibited water intake (Fig. 2*a*). The effect was short lasting and, again, required large amounts of peptide (200 μg per rat). As before, AEN was more effective than LEN and MEN: 10 μg completely inhibited drinking for more than 60 min, and a clear-cut inhibitory effect, lasting more than 30 min, was elicited by doses as low as 100 ng (Fig. 2*b*). Lower doses (10 ng) were still effective, but difference from controls was not statistically significant.

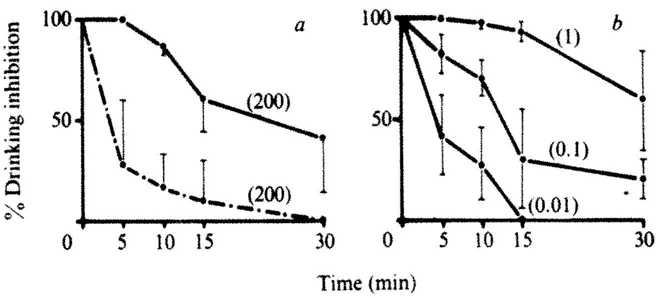

Fig. 2 Water-deprived rats. Inhibitory effects of (Leu^5)-enkephalin (*a*, continuous lines), (Met^5)-enkephalin (*a*, dotted lines) or (D-Ala^2, D-Leu^5)-enkephalin (*b*) on water intake induced by a 16-h period of water deprivation. In parentheses, doses in μg. Points and vertical bars are means ± s.e. of 6–8 experiments.

In both kinds of experiments, animals which received LEN or MEN, 200 μg per rat, were immobile and did not react promptly to tactile and acoustic stimuli. This 'depressive' effect lasted about 5 min and disappeared before the antidipsogenic effect. Rats which received these substances at relatively low doses (25–50 μg) as well as AEN at doses of 1 μg or less did not react in this way. At these dose levels, enkephalins did not produce any nonspecific behavioural modification or modify food intake in rats trained to eat their daily allowance of food during a 2-h period, between 2100 and 2300 (Fig. 3). Naloxone, 2,000 μg per kg, subcutaneously, reduced but did not completely abolish the antidipsogenic effect of the peptides.

The present data provide evidence that i.c. administration of enkephalins elicits in the rat an antidipsogenic effect which is potent, reproducible and dose dependent. This effect might be considered specific and not simply due to the depressive activity of enkephalins. In fact, it was evoked even by doses which did not affect the rats' nonspecific behaviour, as in the case of AEN, which was effective at doses as low as 10 ng, and was still evident after the 'depressive' response had disappeared, as in the case of LEN, 200 μg, which, in water-deprived rats, significantly inhibited drinking for more than 30 min. Moreover, water intake, but not food intake, was modified by the peptides.

The different potency of the naturally occurring enkephalins, on the one hand, and AEN, on the other, may be accounted for by the different sensitivity of the latter to enzymatic hydrolysis, as a consequence of replacing Gly_2 by D-alanine[6].

The results of these experiments, although confirming our working hypothesis, give us no conclusive indication of whether substance P inhibits drinking by means of enkephalin release, nor any indication of the possible physiological significance of the antidipsogenic effect of enkephalins.

Thirst is an unpleasant sensation which may be so severe as to be regarded as a suffering and, in a wide sense, as pain. Thus, it is

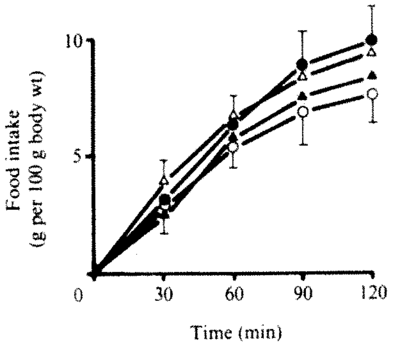

Fig. 3 Food intake in rats trained to eat their daily allowance of food during a 2-h period, between 2100 and 2300 (ref. 1). Controls (▲) received 1 μl of Tyrode solution, i.c.; treated rats received 1 μl of Tyrode solution containing fully antidipsogenic doses of LEN (●, 200 μg), MEN (○, 200 μg) or AEN (△, 1 μg). Points and vertical bars are means ± s.e. of 5–7 experiments.

not entirely impossible that the analgesic effect of enkephalins may be involved in the mechanism(s) of water intake control. However, the fact that the antidipsogenic effect of enkephalins is reduced but not abolished by naloxone suggests that it is only in part, if at all, due to activation of opiate receptors.

These are entirely speculative hypotheses. Experiments are being carried out in our laboratory to check their validity.

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Hyperpolarisation and depression of slow synaptic inhibition by enkephalin in frog sympathetic ganglion

EVIDENCE is accumulating to support the concept that the pentapeptides Met-enkephalin and Leu-enkephalin, the endogenous ligands for the opiate receptor, function as neuromodulators or neurotransmitters[1,2]. In the central nervous system the effects of enkephalins are complex and difficult to analyse, and their mode of action at the neuronal membrane level is still poorly understood. The most prominent action of enkephalins in the mammalian brain is depression of neuronal firing rate and it has been suggested that these peptides are inhibitory transmitters[1,2]. An increase in firing rate by enkephalins has, however, also been reported[3]. The response of central neurones to several putative transmitter substances is depressed or enhanced by enkephalins, suggesting a postsynaptic action[4,5]. It has also been shown that enkephalins suppress the K^+-induced release of noradrenaline[6], dopamine[7] and acetylcholine[7] from rat brain slices, indicating a presynaptic effect. The firing of myenteric neurones in the guinea-pig ileum is inhibited by enkephalins. This inhibition is probably due to a direct postsynaptic action of the enkephalins resulting in a hyperpolarisation of the neuronal membrane[8]. Most of the effects of enkephalins are antagonised by the specific opiate antagonist naloxone. Morphine produces effects very similar to those of enkephalins. Because of the possible role of enkephalins in inhibitory synaptic transmission, we studied the effects of Met-enkephalin on slow synaptic inhibition in frog sympathetic ganglion. We report here that Met-enkephalin causes a depression of the slow inhibitory postsynaptic potential (i.p.s.p.), probably mainly by a presynaptic action, and induces a hyperpolarisation of the ganglionic neurones. Both effects are antagonised by naloxone.

The experiments were carried out on the 9th or 10th paravertebral sympathetic ganglion of the frog (*Rana temporaria* and *R. esculenta*), using the sucrose gap technique[9]. Slow i.p.s.ps were elicited by repetitive stimulation of the ramus to the 8th ganglion. The simultaneously evoked fast excitatory postsynaptic potentials were selectively blocked by a low concentration of nicotine (30 μM) which was continuously present in the perfusion solution. Drugs were applied by switching from the original perfusion solution to one which differed only in its content of the drug to be studied. To detect changes in the ganglionic neurone resting membrane potential, which is reflected by the voltage across the sucrose gap, much care was taken to ensure a stable recording. In most experiments d.c. stability was better than $1\ \mathrm{mV\ h^{-1}}$.

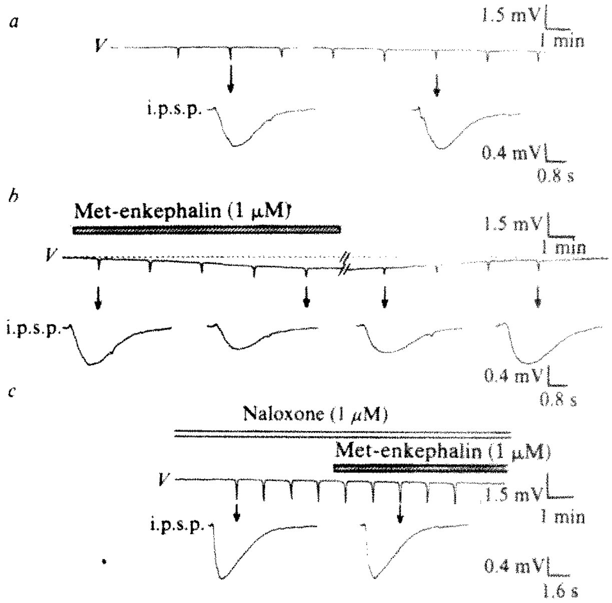

Fig. 1 Effects of Met-enkephalin on membrane potential and slow inhibitory postsynaptic potential (i.p.s.p.) in frog sympathetic ganglion. Experiments were carried out on the 9th or 10th paravertebral sympathetic ganglion using the sucrose gap technique[9]. The ganglion was continuously perfused with Ringer solution, with or without drug, containing in mM: NaCl 160; KCl 2.4; $CaCl_2$ 1.7 and HEPES 3.0; at *p*H 7.3 and temperature 21–23 °C. The postganglionic nerve trunk was perfused with isotonic KCl. Slow i.p.s.ps were elicited by repetitive stimulation of the ramus to the 8th ganglion through a suction electrode with a train of five pulses (0.1 ms; 10 Hz). Fast excitatory postsynaptic potentials were selectively blocked by the continuous presence of 30 μM nicotine in the perfusion solution. Upper trace in *a*, *b* and *c* represents continuous recording of voltage across sucrose gap (*V*) on the strip chart recorder. Downward deflections are slow i.p.s.ps. Lower traces, samples of enlarged slow i.p.s.ps recorded with a faster time base at different times as indicated by arrows. *a*, Control; *b*, hyperpolarisation and depression of slow i.p.s.p. by application of 1 μM Met-enkephalin as indicated by hatched bar. Dashed line in upper trace indicates baseline voltage. Both effects were reversed rapidly when the application of Met-enkephalin was stopped. *c*, Application of 1 μM naloxone (open bar) completely prevented the effects of subsequent application of 1 μM Met-enkephalin (hatched bar). Naloxone itself was without noticeable effect.

Application of 1 μM Met-enkephalin consistently caused a hyperpolarisation of the ganglionic neurones. In all experiments with Met-enkephalin a small drop in voltage across the sucrose gap (0.2–0.5 mV) was observed (Fig. 1*b*). Simultaneously with the hyperpolarisation, Met-enkephalin produced a marked depression of slow synaptic inhibition (Fig. 1*b*). In six experiments the amplitude of the slow i.p.s.p. was depressed by 39.3 ± 6.5% (mean ± s.d.). Duration and time course of the slow i.p.s.p. were not significantly affected by Met-enkephalin. Both the hyperpolarisation and the depression of the slow i.p.s.p. attained a constant level 5–10 min after the enkephalin had reached the ganglion, and were completely reversed on washing with enkephalin-free solution. Reapplication of Met-enkephalin produced the same effects.

D-Ala-Met-enkephalinamide at 1 μM caused similar effects (three experiments), but no attempt was made to compare the potencies of the two peptides. Morphine at 5 μM also caused a hyperpolarisation together with a depression of the slow i.p.s.p. (three separate experiments). Application of 1 μM naloxone fully reversed or prevented both the hyperpolarisation and the depression of the slow i.p.s.p. induced by Met-enkephalin (Fig.

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1*c*). Naloxone itself had no noticeable effect. Thus, the enkephalin-induced effects can be considered to result from a specific interaction with opiate receptors. In myenteric neurones normorphine also causes a naloxone-reversible membrane hyperpolarisation which is accompanied by a fall in membrane resistance, as demonstrated by intracellular recording[10]. The hyperpolarisation of the sympathetic neurones reported here may be due to a similar mechanism.

There is evidence that the driving force for the slow i.p.s.p. is the difference between the membrane potential, V_m, and the K$^+$ equilibrium potential, E_K (ref. 11). Therefore, the depression of the slow i.p.s.p. by Met-enkephalin could be a consequence of the enkephalin-induced hyperpolarisation of the postsynaptic membrane. To test this possibility the relationship between slow i.p.s.p. amplitude and membrane potential was studied by varying the external K$^+$ concentration. The slow i.p.s.p. amplitude seemed to be linearly related to $V_m - E_K$ calculated with the aid of the Goldman equation, assuming standard values for the internal K$^+$ concentration (144 mM) and for the permeability ratio (P_{Na}/P_K, 0.04) (ref. 12). The estimated hyperpolarisation necessary to reduce the slow i.p.s.p. by 40% was much larger than the hyperpolarisation induced by Met-enkephalin, even if it is assumed that the enkephalin affects only C cells which generate the slow i.p.s.p. Thus, an additional effect seems to be involved.

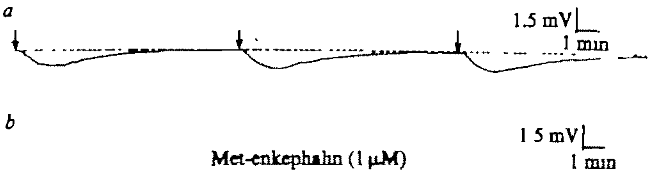

Fig. 2 Effects of Met-enkephalin on transient hyperpolarisations produced by single applications of dopamine (1 μl, 100 mM) just over the ganglion (arrows). *a*, Control; *b*, after application of 1 μM Met-enkephalin as indicated by hatched bar. Dashed line indicates baseline voltage across sucrose gap. Asterisk denotes beginning of hyperpolarisation induced by Met-enkephalin.

The slow i.p.s.p. in sympathetic ganglia is considered to be brought about by a catecholamine, probably dopamine, released from an interneurone which in turn is activated through a muscarinic cholinergic synapse[13]. To determine whether Met-enkephalin influenced the sensitivity of the ganglion to dopamine, small amounts of this substance were applied just over the ganglion with the aid of a motor-driven microinjection device. A single ejection of 1 μl of a 100 mM solution of dopamine produced a transient hyperpolarisation with a maximum amplitude of 0.5–3.0 mV in different experiments (Fig. 2*a*). Repeating the ejections at an interval of 10 min produced a constant response over a period of hours. Application of Met-enkephalin (1 μM) had only a slight effect on the dopamine response (Fig. 2*b*); in five experiments the amplitude was reduced by an average of 13.8 ± 6.1%. This reduction may be caused by the enkephalin-induced hyperpolarisation of the postsynaptic membrane as discussed above. These results suggest that the postsynaptic sensitivity of the ganglionic cells towards dopamine is hardly affected by Met-enkephalin.

The most likely explanation of the depression of the slow i.p.s.p. by Met-enkephalin reported here is an inhibition of transmitter release from presynaptic nerve terminals. Such a mechanism has been postulated to explain the inhibition of the K$^+$-induced release of various putative transmitter substances, including dopamine, from rat brain slices by enkephalin[6,7]; a presynaptic localisation of opiate receptors on dopaminergic nerve terminals has been demonstrated in rat striatum[14].

In conclusion, the present results show that Met-enkephalin exerts a dual effect in frog sympathetic ganglion; it induces a hyperpolarisation of the ganglionic neurones and in addition depresses slow synaptic inhibition. The hyperpolarisation probably involves a postsynaptic site of action, whereas the depression of slow synaptic inhibition may be presynaptic in origin, due to the inhibition of transmitter release from the nerve terminals.

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mRNA species directing synthesis of milk proteins in normal and tumour tissue from human mammary gland

ONLY about one third of human metastatic breast carcinomas regress in response to hormone therapy[1,2], so there is a need to predict which tumours are of the hormone-dependent type. The usual method of prediction is based on the determination of hormone receptors in the tumour tissue[2–5]. However, only about one half of the tumours containing measurable oestrogen-receptor levels respond to hormone treatment[2] suggesting that receptors which bind the hormone *in vitro* may not always respond *in vivo*. An alternative approach has been to look for milk proteins within the tumour or serum, as lactation is a known hormone-dependent process. Although the rationale behind this approach has been substantiated in experimental systems[6,7] results with human mammary carcinomas are inconclusive[8–16], probably because of insufficient specificity[17] and sensitivity of the radioimmunological protein assay on which this method depends. In addition to technical problems the immunological approach has the drawback that it can only monitor the final product of hormone action, the mature protein, and not the initial RNA transcript(s). A better approach would seem to be an assay designed to detect differences in transcriptional rather than translational activity. As a preliminary step towards this objective, we describe here the isolation of poly(A)-containing RNA from a normal lactating human mammary gland, and demonstrate the presence of mRNA species which direct the synthesis in cell-free protein-synthesising systems of human casein and α-lactalbumin. Moreover, using the same techniques we have identified the presence of mRNA directing the synthesis of α-lactalbumin in poly(A)-containing RNA isolated from two out of five mammary tumours examined.

The tissue we describe as 'normal' was obtained from a nursing mother 15 days postpartum and consisted of a large (2 g) retention cyst, histologically virtually normal, which showed

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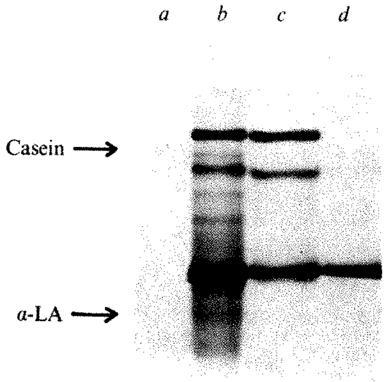

Fig. 1 Identification of casein and α-lactalbumin (α-LA) mRNAs in poly(A)-containing RNA isolated from lactating human mammary gland. A 2-g sample of frozen human lactating mammary gland (−70 °C) was pulverised with a hammer, then ground to a coarse powder in a mortar and pestle with periodic addition of liquid nitrogen. The powdered tissue was then rapidly transferred to a Teflon-in-glass Potter–Elvehjem homogeniser (0.4 mm clearance) containing 20 ml of 2% (w/v) SDS, 0.5 mg ml^{-1} proteinase K (Boehringer), 1 mM EDTA, 20 mM Tris-HCl, *p*H 7.6 and homogenised briefly at room temperature. It was then incubated at 45 °C for 1 h. After adding NaCl to 0.1 M, the lysate was extracted once with an equal volume of phenol–chloroform (1 : 1) saturated with 0.1 M NaCl, 1 mM EDTA, 20 mM Tris-HCl, *p*H 7.6. After separating the phases by centrifugation (3,000*g*, 10 min, 15 °C) nucleic acids were precipitated from the aqueous phase with 2.5 volumes of absolute ethanol at −20 °C for 1 h. The precipitate was collected by centrifugation (12,000*g*, 10 min, 0 °C) and redissolved in 11 ml of 1 mM EDTA, 20 mM Tris-HCl, *p*H 7.6. Solid caesium chloride (4.4 g) was added (0.4 g ml^{-1}) and the solution was distributed between two 12-ml capacity Beckman Ti50 centrifuge tubes, each containing a 3-ml cushion of 5.7 M caesium chloride in buffer. After overlaying with paraffin oil the tubes were centrifuged at 40,000 r.p.m. for 18 h at 20 °C in a Beckman Ti50 rotor. The supernatant was removed, the bottoms of the tubes sliced off, and the RNA pellets redissolved in buffer. After ethanol precipitation, the total RNA was subjected to two cycles of affinity chromatography on oligo(dT)-cellulose[18] to obtain the poly(A)-containing fraction. mRNA activity was determined in a wheatgerm cell-free protein-synthesising system[18], in the presence of ^{35}S-methionine. The products were electrophoresed on Maizel polyacrylamide gels[31] containing SDS[32], and visualised by fluorography[33]. Antisera to human α-lactalbumin and the major casein isolated from milk[9,20,34] were raised in rabbits[18]. Immunoprecipitates were prepared and processed as described previously[18]. *a*, Wheatgerm products synthesised in the absence of added RNA. *b*, Proteins synthesised on the addition of poly(A)-containing RNA from lactating human mammary gland. *c*, Proteins specifically precipitated by combined antisera to human α-lactalbumin and the major casein isolated from milk. *d*, Proteins specifically precipitated by anti-α-lactalbumin antiserum alone. Arrows indicate the positions of authentic mature milk proteins isolated from milk.

lactating breast tissue with areas of severe inflammation and abscess formation, but no evidence of malignancy. This and other tumour tissue samples were deep-frozen in liquid nitrogen within minutes of excision, and then stored at −70 °C before processing as described in the legend to Fig. 1.

The addition of the total cellular poly(A)-containing RNA isolated from normal human lactating mammary gland tissue to the wheatgerm cell-free protein-synthesising system resulted in a fourfold stimulation in the incorporation of ^{35}S-methionine into protein, when compared with that of the endogenous level in the absence of added RNA. Visualisation of the synthesised products by fluorography after separation by SDS-gel electrophoresis revealed the presence of three prominent bands of estimated molecular weights 26,500, 23,000 and 16,500 (Fig. 1). However, none of these products co-electrophoresed with the authentic human milk proteins (casein 25,000; α-lactalbumin 14,500), a situation we have previously reported with respect to the mRNA-directed synthesis of guinea pig milk proteins in the wheatgerm cell-free protein-synthesising system[18]. Further product analysis, using a mixture of antibodies raised against highly purified preparations of the major human casein component, and of human α-lactalbumin, resulted in the preferential precipitation of all three bands, whereas treatment with antisera raised against α-lactalbumin alone resulted in the precipitation of the lowest molecular weight component only (Fig. 1).

These results are consistent with the synthesis in the wheatgerm cell-free protein-synthesising system (or the reticulocyte lysate system—L. H. and R. C., unpublished observations) of a precursor form of α-lactalbumin (pre-α-lactalbumin). The situation concerning the remaining two higher molecular weight protein products is less clear. Human milk contains two caseins, the major component a well characterised protein (molecular weight 25,000), and a minor component, the κ-casein. We have purified and raised antisera against the former, although it is known that this cross-reacts immunologically with the κ-casein component[19]. Thus it seems reasonable to conclude that both bands represent human caseins, and that the higher molecular weight predominant protein probably represents a precursor to the major human casein, whilst the minor lower molecular weight protein probably represents the equivalent precursor to the κ-casein. However, as it is known that the major human casein is also highly phosphorylated[20], one cannot exclude the possibility that the anomalous mobility of the human caseins might be due to the absence of this post-translational event in the wheatgerm cell-free system. The relevance of pre-proteins and the absence of post-translation modification events in the wheatgerm cell-free system, concern the intracellular mechanisms involved in the synthesis of secretory proteins. These have been discussed in detail elsewhere with regards to guinea pig milk protein synthesis[18,21] and will not be discussed further in the context of this paper, except to note that in common with the guinea pig[18], rat[22], ovine and bovine milk proteins[23,24], the primary human milk protein translational products are probably synthesised as pre-proteins.

Processing of human mammary tumour tissue in an identical manner produced considerably lower yields of total RNA per g of starting material (15 A_{258} units per g) than the normal lactating tissue (75 A_{258} per g), although in all tissue samples processed in this manner the poly(A)-containing RNA recovered consistently represented about 1% of the total RNA population. Addition of the tumour-derived poly(A)-containing RNA to the wheatgerm cell-free system resulted in a slight stimulation in protein synthesising activity in two out of five tumours examined (all from postmenopausal patients). Analysis of the *in vitro* synthesised ^{35}S-methionine-labelled protein

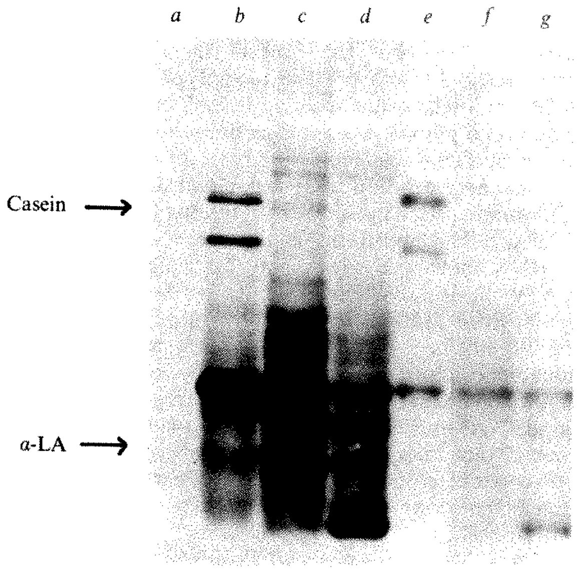

Fig. 2 Identification of α-lactalbumin mRNA in poly(A)-containing RNA isolated from human breast tumours. Experimental details as for Fig. 1, poly(A)-containing RNA being isolated from frozen breast tumours in the way described for lactating tissue. *a*, Wheatgerm products, synthesised in the absence of added RNA. *b–d*, Proteins synthesised on the addition of poly(A)-containing RNA from lactating human mammary gland (*b*), breast tumour 1 (*c*) and breast tumour 2 (*d*). *e–g*, Proteins specifically precipitated by combined antisera to human α-lactalbumin and the major casein isolated from milk; lactating human mammary gland (*e*), breast tumour 1 (*f*) and breast tumour 2 (*g*). Arrows indicate the positions of authentic mature milk proteins isolated from milk.

products (Fig. 2) showed in each case a prominent protein band which co-electrophoresed with human pre-α-lactalbumin, but no comparable bands which co-electrophoresed with the equivalent putative human pre-caseins. Antibody precipitation of the products with a mixture of antisera against both human α-lactalbumin and the major casein component confirmed that neither tumour contained significant levels of active mRNA which directed the synthesis of human caseins, though both contained mRNA which directed the *in vitro* synthesis of pre-α-lactalbumin. In addition to pre-α-lactalbumin, poly(A)-containing RNA from tumour 2 also directed the synthesis of a prominent lower molecular weight protein product (11,500), also precipitable by milk-protein antibodies. Subsequent work has shown that this is precipitated by antisera raised against α-lactalbumin, and not by antisera raised against human casein preparations.

Of the remaining three mammary tumours processed, although poly(A)-containing RNA was recovered in the expected yield, all showed inhibitory activity in a variety of cell-free protein-synthesising systems, possibly a function of low molecular weight nuclear RNA species known to inhibit cell-free protein-synthesising systems[25–28].

As well as demonstrating mRNA-directed synthesis *in vitro* of human milk proteins, our results provide firm evidence for the presence, in significant levels, of mRNA directing the synthesis of α-lactalbumin but not casein, in two out of five mammary tumours examined. Both were from postmenopausal patients, one of which (T1—an adenocarcinoma) had had no children, whilst the other (T2—an invasive duct carcinoma) had had one breast-fed child. Neither had undergone either hormone therapy or chemotherapy before surgery.

α-Lactalbumin has previously been identified in the cytosol fraction of mammary tumours, using radioimmunoassay. One study in particular[16] provided good evidence for the presence of α-lactalbumin in 38% of all mammary carcinomas examined. Of these, 53% of the ER-positive carcinomas contained α-lactalbumin, but only 5% of the ER-negative carcinomas, results which strongly suggest that the presence of α-lactalbumin may indicate an intact oestrogen-receptor mechanism, and may therefore provide a useful marker for identifying tumours responsive to hormone therapy. Our preliminary results support such a concept. Our approach is at present time-consuming and therefore not suitable for routine diagnostic application. However, it should prove possible to apply modern recombinant genetic techniques to this system, as has been done with both adult and fetal globin genes[29], thus providing in the form of a recombinant DNA molecule the basis of a highly sensitive nucleic acid hybridisation assay capable of detecting low levels of milk protein mRNA sequences in relatively small samples of breast tissue. This approach has the additional advantage that other RNA molecules which may be inhibitory in cell-free protein synthesising systems will have no deleterious effect on molecular hybridisation. The potential of molecular hybridisation has already been demonstrated by Rosen and Socher[30] who examined the effect of hormones on the levels of rat casein mRNA by hybridisation to a complementary DNA copy of the casein mRNA, thus by-passing any inconsistencies in radioimmunoassay technology, and the dependence of such assays on the presence of the final translational product, and not the initial product of hormone-dependent gene expression, the primary RNA transcript.

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Translocation 8;14 in an ataxia telangiectasia-derived cell line

THE incidence of lymphomas is 10,000 times greater than normal in patients with immunodeficiency syndromes[1] and also in ataxia telangiectasia (AT), which very often involves IgA deficiency[2]. In addition to its other characteristic features, AT is usually associated with a defect in DNA repair[3] and a translocation involving chromosome 14 in the peripheral leukocytes[4]. Another feature is a high prevalence of Epstein–Barr virus early antigen (EBV-EA) antibodies[5] in the sera of AT patients. This is significantly higher than in normal human sera (<10%) and resembles that found in cases of Burkitt's lymphoma (BL). It is thus interesting that translocations involving chromosome 14 have been described in BL cells as well as in other lymphomatous cells[6–8]. This chromosome marker is also found in BL cell lines containing EBV[9]. It is not induced by EBV alone, however, because it is not found in EBV-positive, but non-lymphomatous cell lines, established spontaneously or by transformation of normal peripheral blood leukocytes[10]. We have therefore investigated whether in BL and AT some target cells of EBV infection might be genetically 'defective' lymphoid cells that could more easily be transformed into malignant cells (with characteristic chromosome markers) when exposed to EBV or other carcinogens. To do this we infected lymphoid cells from AT patients *in vitro* with EBV, and we report here a translocation $t(8q^-; 14q^+)$ established in one of the resulting cell lines.

The translocation seems to be identical to that found in BL[6,7]. This suggests that *in vitro* transformation of peripheral lymphocytes from a patient with a genetic predisposition to

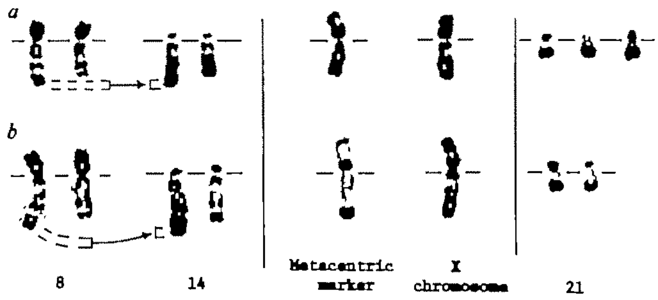

Fig. 1 R-banded chromosome rearrangements in *a*, ataxia telangiectasia- *b*, Burkitt lymphoma-derived cell lines

chromosome damage favours the development of a chromosome rearrangement already described in lymphoid malignancies[8,10].

Phytohaemagglutinin-stimulated peripheral blood lymphocytes from a 17-yr-old male patient with AT were analysed by R-banding. In addition, a cell line (CB-1) was established from his blood lymphocytes by transformation *in vitro* with EBV obtained from the saliva of a patient with infectious mononucleosis according to a method previously described[11]. Karyotypes were prepared from this cell line 6, 8 and 12 months after it had been established. Structural rearrangements were compared with those seen in a BL-derived cell line (HR1K-m) which was examined on two separate occasions, 1 month apart, and with those of a control lymphoblastoid cell line established from normal cord blood lymphocytes transformed by EBV (Cb 8-11). Chromosomes of the three cell lines were analysed using a modification of the original technique of Dutrillaux and Lejeune[12]. R-bands obtained with this technique reveal abnormalities involving relatively small portions of chromosomes.

No significant chromosomal abnormalities were observed in 108 metaphases from the peripheral blood leukocytes of the patient. However, translocations involving 14q have been described in the blood of some other AT patients[4]. The major part of 14q (break point in band 14q12) is translocated to the end of the homologue 14 or chromosomes 6, 7 or X (ref. 4). As Fig. 1 shows, an AT-derived cell line shows three different chromosomal abnormalities: a translocation $t(8q^-;\ 14q^+)$, a metacentric marker of the size of an X chromosome with a large dark band just above the centromere, and a trisomy 21. The first two abnormalities closely resemble those seen in the BL-derived cell lines, Burkitt tumour cells and other lymphomatous cells. In the control cell line (Cb 8-11) derived from normal cord blood lymphocytes transformed by EBV, chromosomes 8, 14 and 21 were normal and no metacentric marker was seen. All 35 metaphases from the AT-derived cell line contain the translocation t8;14 and the metacentric marker (Table 1). Trisomy 21 is absent only from three metaphases with 47 chromosomes. This could be interpreted as either the presence of a second clone or the loss of a G chromosome. It is interesting that although the patient had no features of the trisomy 21 syndrome, two first-degree cousins of his father had given birth to children with Down's syndrome.

Table 1 Chromosome markers in ataxia telangiectasia- and Burkitt lymphoma-derived cell lines

Cell line origin	Photographed metaphases Total no.	No containing a t(8,14)	 Metacentric marker	 Trisomy 21
AT+EBV	35	35	35	32
BL	36	36	36	0

AT, Ataxia-telangiectasia; BL, Burkitt lymphoma (cell line HRI-Km); EBV, Epstein–Barr virus.

EBV can transform peripheral blood lymphocytes from normal donors into permanent cell lines which remain diploid for a long time after establishment. Non-random chromosome abnormalities are acquired on prolonged culture. After more than a year, gains particularly involving five autosomes (numbers 3, 7, 8, 9 and 12) and sex chromosomes have been reported in these lymphoblastoid cell lines[13]. In our investigation, chromosome rearrangements similar to those seen in BL-derived cell lines were already present at the time of the first cytogenetic analysis, 6 months after EBV transformation.

Because both cell lines come from male donors, the possibility of contamination of one cell line by another cannot be excluded by using the Y chromosome as a distinctive marker. However, they have different histocompatibility antigens (CB-1: A3AW32, B5BW35, CW2; HR1K-m: A28AW34, B15BW49, CW2). Although an unusually high incidence of chromosomal damage[14] and defective DNA repair[3] has been reported in AT skin fibroblasts, rates of SV40 transformation of these cells are no higher than those for normal cells[15]. Furthermore, marker chromosomes similar to that described here have not been identified in these fibroblasts. In our experiment, AT lymphocytes were found, after transformation *in vitro*, to have a 14q translocation which seems to be a marker of lymphoid malignancy[4,7,8,16].

It seems that the addition of EBV to apparently normal lymphocytes of an AT patient has revealed either a quiescent clone or an increased chromosome fragility. This is interesting because EBV transformation of lymphoid cells from normal individuals has not so far induced the characteristic chromosome 14 marker seen in lymphomatous cells[10]. Further studies will be necessary to characterise CB-1 as a 'lymphomatous cell line' or as a 'lymphoblastoid cell line' as proposed by Nilsson[17]. The role of genetic factors such as defective DNA repair, and of susceptibility to oncogenic agents in cell transformation and malignancy may thus be clarified. Cell lines derived from lymphocytes of AT patients and others with increased susceptibility to lymphoid malignancy, might become useful tools for the study of malignant transformation. Lymphocytes from other AT patients are now being transformed by EBV, and cytogenetic studies will be made to discover if a correlation exists between early chromosome rearrangements and future clinical course, with special attention to the development of lymphomas.

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Re-evaluation of the number of specific β-adrenergic receptors on muscle cells

IT has been reported by Atlas, Hanski and Levitzki that muscle cells have an unusually high number of β adrenoreceptors, 80,000 per cell[1]. However, we consider the pharmacological characterisation of these receptors as specific β adrenoreceptors unsatisfactory. We report here that the number of specific β adrenoreceptors in a similar but not identical muscle cell line of the same origin is 8,000 rather than their stated figure of 80,000, and is therefore comparable to that found on C6 glioma cells.

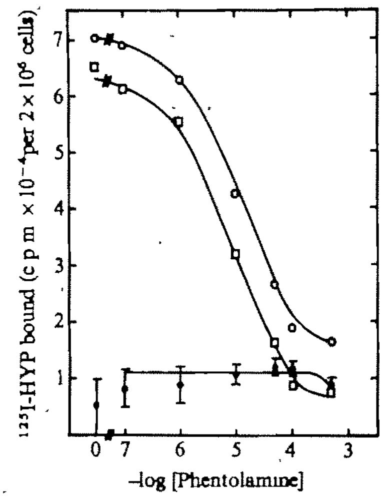


Fig. 1 The effects of phentolamine on ^{125}I-HYP binding to intact L8 muscle cells. An L8 cell line was established by serial passage of rat myoblasts[6]; it has properties similar to those of the L6P cell line. The cells were grown on Falcon or Nunc tissue cultures plates in Dulbecco's minimal essential medium (Gibco) supplemented with 10% fetal calf serum (Gibco), under an atmosphere of 95% air, 5% CO_2. Trypsinised, exponentially growing cells were distributed on to 6-cm plastic plates. They were then allowed to grow and recover for 48 h in fresh medium before the binding assay. In this condition the cells multiplied about ninefold. This means that if trypsin has any effect on the β-receptor density, it would affect only 10% of the cells. HYP was iodinated by the method of Maguire *et al.*[4]. 2×10^6 cells per 6-cm plate were incubated with ^{125}I-HYP (125,000 c.p.m. or 25 pM) in 2 ml of phosphate-buffered saline (PBS) and various concentrations of phentolamine in the absence (○) or presence (□) of 10^{-7} M (–)propranolol Prewashing of the plates, their incubation with the radioactive ligand and further washing were carried out as previously described[2]. The washing covers a total period of 60 min at 37 °C. Such a prolonged washing has negligible influence on the dissociation of ^{125}I-HYP specifically bound but markedly decreases the nonspecific binding (not shown). This observation is compatible with what is found in the literature concerning ^{125}I-HYP binding. The dissociation of ^{125}I-HYP specifically bound is observed only when an excess of cold antagonist is added during the washing[4,7]. In the absence of excess cold antagonist, binding seems to be virtually irreversible. The amount of specifically bound (●) ^{125}I-HYP is the calculated difference between total (○) and nonspecific binding (□).

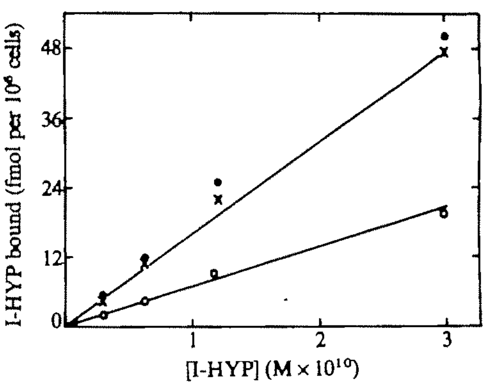


Fig. 2 ^{125}I-HYP binding to L8 muscle cells in the absence of phentolamine. About 2×10^6 cells per 6-cm plate were incubated with 2 ml of PBS containing various concentrations of ^{125}I-HYP, in the absence of phentolamine, without propranolol (●) and in the presence of 2.5×10^{-5} M (○) or 10^{-7} M(×) (–)propranolol and assayed for binding as described in Fig. 1.

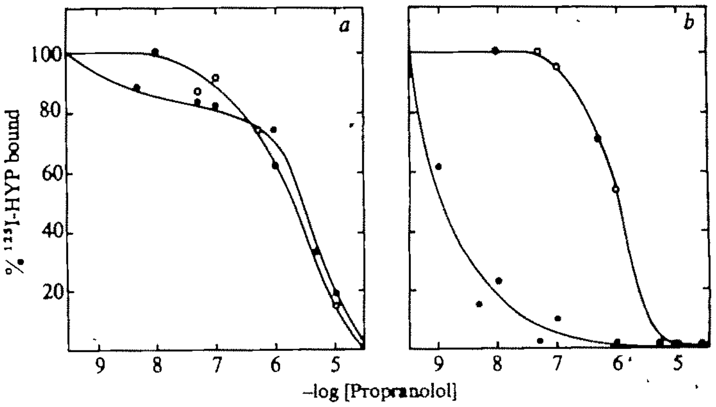
We have recently devised a method to measure the number of β adrenoreceptors on intact C6 glioma cells[2]. That study demonstrated that tissue culture cells bind large amounts of ^{125}I-hydroxybenzylpindolol (^{125}I-HYP) in a nonspecific manner. This nonspecific binding can be largely eliminated by carrying out the binding assays in the presence of high concentrations of phentolamine as originally observed by Sporn and Molinoff[3] in membranes from rat brain. Figure 1 shows that 5×10^{-5} M phentolamine displaces 75% of the nonspecifically bound ^{125}I-HYP but does not modify the specifically bound ligand.

In addition, the concentration of cold competing antagonist or agonist used to measure the nonspecific binding must be chosen according to its dissociation constant and in relation to the concentration of radioactive ligand. In our study, 10^{-7} M (–)propranolol was a satisfactory concentration ($100\times K_D$). Higher concentrations are not necessary to displace ^{125}I-HYP bound to β receptors and such high concentrations can displace ^{125}I-HYP bound nonspecifically.

Figure 2 shows the difference in two nonspecific binding curves obtained on L8 muscle cells in the presence of two different concentrations of (–)propranolol, 2.5×10^{-5} M and 10^{-7} M in the absence of phentolamine. The difference between these two curves represents displacement of nonspecifically bound radioactive ligand.

This conclusion is supported by the results shown in Fig. 3. 10^{-7} M (–)propranolol, the pharmacologically active optical

Fig. 3 Inhibition of ^{125}I-HYP binding to L8 muscle cells by propranolol. Binding was carried out as described in Fig. 1, in the absence (*a*) or presence (*b*) of 5×10^{-5} M phentolamine, with (+)propranolol (○) or (–)propranolol (●). 100% and 0% binding refer, respectively, in *a* to 100,000 and 30,000 c.p m. and in *b* to 40,000 and 22,000 c p m. These experiments were done in parallel on the same number of cells and the results can thus be compared quantitatively as well as qualitatively.



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isomer, provokes nearly maximal displacement from stereospecific sites. The reduction in binding observed in the absence of phentolamine with concentrations of propranolol higher than 10^{-7} M is clearly non-stereospecific and thus represents displacement from nonspecific sites. Figure 3*b*, in which most of the nonspecific ^{125}I-HYP bound has been removed in the presence of phentolamine, strikingly illustrates the stereospecific displacement from β receptors. Atlas *et al.* carried out their assays with similar concentrations of radioactive ligand but in the absence of phentolamine. In these conditions, their concentration of ($\pm$)propranolol, 5×10^{-5} M, was two orders of magnitude too high and displaced ^{125}I-HYP bound to sites unrelated to β-adrenergic receptors. It should be noted that the relative amount of nonspecifically bound ^{125}I-HYP is highly variable among different cell lines. In turkey erythrocytes, nonspecific binding is much lower and thus the use of 10^{-7} M or 5×10^{-5} M ($\pm$)propranolol would not change the amount of displaced ^{125}I-HYP in such a dramatic fashion. In Fig. 4, the specific binding of ^{125}I-HYP to β adrenoreceptors has been measured in the presence of phentolamine using 10^{-7} M ($-$)propranolol as the competing ligand. Scatchard analysis of these data shows that L8 muscle cells have 7,900 ± 1,600 specific β adrenoreceptors per cell. This number is not significantly different from that obtained in C6 glioma cells[2,4,5]. The use of 2.5×10^{-5} M ($-$)propranolol (Fig. 2) without phentolamine gives an erroneously high number of receptors per cell, thus explaining the figure given by Atlas *et al.*

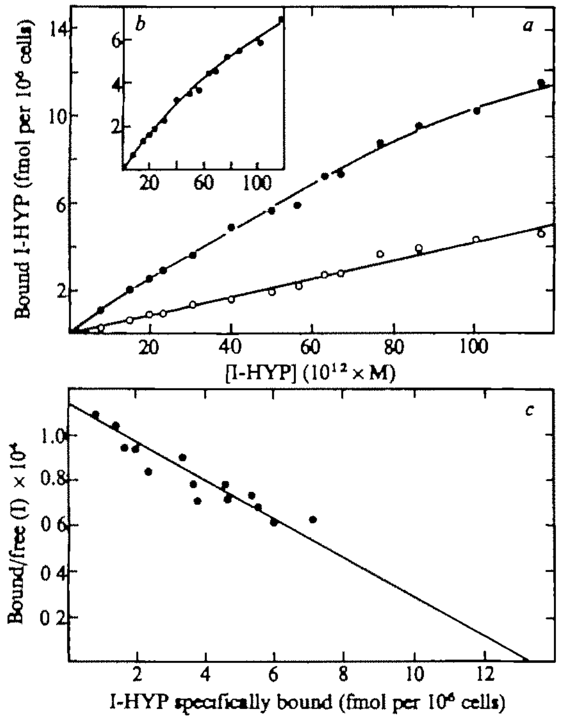

Fig. 4 Binding of ^{125}I-HYP to intact L8 muscle cells in the presence of phentolamine. Assays were carried out in standard conditions. 3×10^{6} cells per plate were incubated with 5×10^{-5} M phentolamine and various concentrations of ^{125}I-HYP. In *a*, the total amount of ^{125}I-HYP bound (●) and the nonspecifically bound ligand remaining in the presence of 10^{-7} M ($-$)propranolol (○) are plotted as a function of the ^{125}I-HYP. Specific binding, defined as the difference between the total and the nonspecific binding, is plotted in *b*. The saturation value (13.2 fmol per 10^{6} cells or 7,950 receptors per cell) and the K_D (1.2×10^{-10} M) has been calculated using a linear regression curve (coefficient of correlation = 0.92) obtained with the Scatchard plot represented in *c*.

Our results demonstrate the importance of the choice of experimental conditions in the determination of the true number of stereospecific β receptors. Although the number of β adrenoreceptors on rat muscle cells of the L8 or L6 line does not single them out for receptor purification, it is, however, sufficient to analyse β-adrenoreceptor distribution during muscle cell fusion.

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ATLAS, HANSKI AND LEVITZKI REPLY—Our reply will be divided into two parts: first, the validation of our results[1], and second, the demonstration that the methodology used by Pochet and Schmitt is inadequate.

To consider our first point. The difference between the binding curve of ^{125}I-($\pm$)hydroxybenzylpindolol (HYP) in the absence and in the presence of 5.0×10^{-5} M ($\pm$)propranolol monitors the reliable number of β adrenoreceptors in intact cells as well as in membrane fragments prepared from these cells. Figure 1 shows Scratchard plots of the binding of ^{125}I-($\pm$)-HYP to L6P cells removed from the growth plate by EDTA as compared with L6P cells removed by using trypsin. It can be seen that trypsin causes a five- to sixfold decrease in the number of β adrenoreceptors. The destruction of receptors by trypsin is well documented for several hormone receptors including β adrenoreceptors. In contrast to the effect of trypsin on the number of β adrenoreceptors detected, no change was observed in the number of ^{125}I($\pm$)HYP binding sites by changing the fluidity of the cell membrane. A large change in the fluidity of the turkey erythrocyte membrane has no effect on the extent of the 'nonspecific' or specific ^{125}I-($\pm$)HYP binding. Table 1 summarises results demonstrating that the ratio between cholesterol and phospholipids has no influence on the binding of ^{125}I-($\pm$)HYP to intact turkey erythrocytes or to membrane fragments prepared from these cells. Further details on this particular point are given elsewhere[2]. The fluidisation of turkey erythrocyte membranes using free fatty acids also has no influence on the number of specific ^{125}I-($\pm$)HYP binding sites on the turkey erythrocyte or on the affinity of these receptors to the β blocker[11].

The number of β adrenoreceptors on a turkey erythrocyte, as measured by ^{125}I-($\pm$)HYP, is identical to the number found by the ^{3}H-($\pm$)propranolol technique introduced by us[3-5]. Furthermore, the measurement of the content of β-adrenergic receptors using ^{3}H-($\pm$)isoprenaline again gives similar results[3]. It is worth noting that the difference between ^{125}I-($\pm$)HYP binding in the absence and in the presence of ($\pm$)propranolol gives linear Scatchard plots, thus indicating that a single class of ^{125}I-($\pm$)HYP binding sites is being monitored. If Pochet and Schmitt were correct in their assertion that ($\pm$)propranolol displaced both specific and nonspecific ^{125}I-($\pm$)HYP, one would

Table 1 Effect of cholesterol enrichment and of cholesterol depletion on the β-adrenergic system

Treatment	Cholesterol to phospholipid ratio	^{125}I-HYP binding (pmol per mg)	^{125}I-HYP receptor dissociation constant
No treatment (control)	1.0–1.2	0.55 ± 0.05	$(6.8\pm0.5)\times10^{-11}$
Cholesterol depleted	0.5–0.6	0.53 ± 0.05	$(7.5\pm0.05)\times10^{-11}$
Cholesterol enriched	1.45–1.55	0.54 ± 0.05	$(6.9\pm0.5)\times10^{-11}$

observe at least two classes of ^{125}I-(±)HYP binding sites which should have given curvilinear Scatchard plots. In our binding studies on intact cells of different types, L6P cells, HeLa cells, turkey erythrocytes and turkey reticulocytes, we have consistently found a single class of ^{125}I-(±)HYP binding sites[2,6]. It should be noted that the successful use of ^{125}I-(±)HYP in the determination of the number of β adrenoreceptors on intact cells was first introduced by Aurbach *et al.*[7] and has since been adopted by several laboratories, including our own. Furthermore, the methodology used by us was originally introduced by Aurbach *et al.*[8] and was demonstrated to be an equilibrium technique[8,9]. Maguire *et al.*[9] have also used propranolol (1.0×10^{-5} M (−)propranolol) for the displacement of the nonspecific ^{125}I-(±)HYP bound, obtaining reliable and consistent binding data.

Schmitt and Pochet are correct in asserting that concentrations lower than 5.0×10^{-5} M (±)propranolol should be sufficient to displace the specific ^{125}I-(±)HYP bound. Indeed, 2.0×10^{-7} M, 2.0×10^{-6} M and 2.0×10^{-5} M (±)propranolol displace bound ^{125}I-(±)HYP to a similar extent. Interestingly, using the usual methodology as described [1,2,6-10], we found that inclusion of 2.5×10^{-5} M phentolamine in the binding mixture reduces the nonspecific binding of ^{125}I-(±)HYP by 10–15% but does not modify the pattern of ^{125}I-(±)HYP to the specific sites. As the effect of phentolamine is marginal (see also below), we find no need to use it. These results are similar to those reported by Brown *et al.* for turkey erythrocytes[10].

On our second point, the methodology used by Pochet and Schmitt suffers from several flaws which cast serious doubts on the validity of their experimental approach. (1) What the authors call 'nonspecific' ^{125}I-(±)HYP binding is removed by six washings, 10 min each, at 37 °C. The methodology used by the authors is cited in their manuscript (their ref. 2). As the half life of ^{125}I-(±)HYP on the β adrenoreceptors is $t_{\frac{1}{2}} = 7.2$ min at 30 °C (ref. 7) and $t_{\frac{1}{2}} = 2.57$ min at 35 °C (ref. 8), it is clear that most of the specific ^{125}I-(±)HYP bound to the β adrenoreceptors is washed out. Therefore, the methodology used is not an equilibrium technique and cannot give reliable data. (2) The cells are removed by trypsin. As we have shown (Fig. 1 and ref. 6), the β adrenoreceptors on L6P cells are destroyed in these conditions. This was the reason EDTA[1,6] was used instead. No evidence is provided by the authors that the 'recovery' period results in the resynthesis of the β adrenoreceptors. (3) The authors use phentolamine to displace nonspecific ^{125}I-(±)HYP binding. At least in one case (rat hypothalamus membranes) phentolamine was found to displace ^{125}I-(±)HYP from β adrenoreceptors[12]. It should be noted that phentolamine, at the concentration range of 1.0×10^{-5} to 1.0×10^{-4} M, can block the isoprenaline-dependent adenylate cyclase activation in neuroblastoma–glyoma hybrids (unpublished data). In our experiments, phentolamine had an insignificant effect. This large variability in phentolamine action on different cells convinces us that its use in binding studies is dispensable. (4) As to the quality of the binding curves presented by Pochet and Schmitt, their Fig. 2 does not show any of the curvature which should be observed if the binding of ^{125}I-(±)HYP has a saturable component. The data presented in their Fig. 4 do not demonstrate that saturation has been achieved. The data can be easily fitted by a straight line. A replot of the raw data in their Fig. 4*a*, according to Scatchard, shows that only 43% saturation has been achieved. If one believes that the remaining 57% behave in a similar fashion, the maximal number of receptors should be 9,650 receptors ($K_D = 1.6\times10^{-10}$ M) per cell. In view of the methodology used by the authors, we believe that this number underestimates the true number of β receptors by about 80–90%. (5) The authors used L8 cells, whereas we used L6P cells.

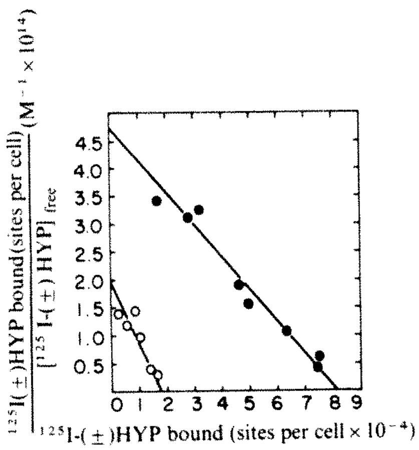

Fig. 1 ●, L6P cells removed by EDTA treatment. Experimental details are given elsewhere[1,6]. ○, L6P cells were removed by treatment with trypsin. The growth medium was removed from the tissue culture bottle and the cells were incubated with 50 ml 130 mM saline, containing 20 mM Tris-HCl buffer, *p*H 7.4, and 500 μg ml^{-1} trypsin, for 30 min at 37 °C. The cells were detached from the bottle, centrifuged at 500 *g*, and washed twice with trypsin-free saline containing buffer. The binding procedure was the same as described elsewhere[1,2,6].

Note added in proof: Recently the density of β-receptors was measured on L84 rat skeletal myogenic muscle cells, using an ultrastructural probe for the receptor. Using this technique receptor density was found[13] to be 71 ± 19 receptors per μm^2. That is, 50,000 to 80,000 receptors per cell—similar to the closely related L6 cells.

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Minor differences between nucleotide sequences of mild and severe strains of potato spindle tuber viroid

VIROIDS are the smallest replicating pathogenic agents known[1]. Composed entirely of RNA, they have genome sizes in the range of 330–380 nucleotides[2], 10 times smaller than the smallest bacteriophage of *Escherichia coli*[3]. Although there is no evidence for the existence of a protective protein coat for viroids, they infect a wide variety of plants and in many produce

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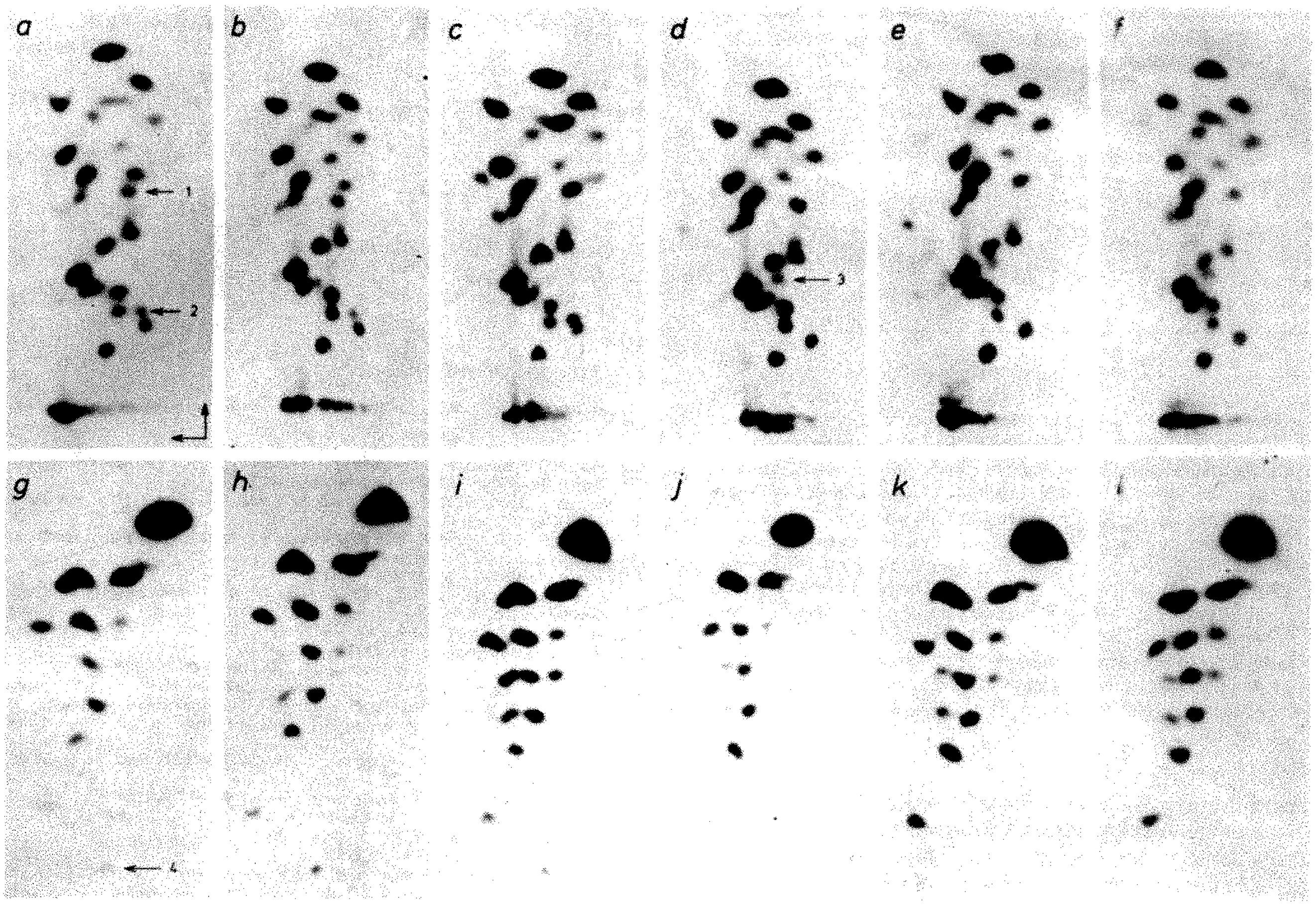

Fig. 1 Ribonuclease T1 and pancreatic ribonuclease fingerprints of two severe, one intermediate and three mild strains of PSTV. 1×10^6 c.p.m. aliquots of each ^{125}I-labelled viroid were dried down in silicone-treated test tubes and digested with 2 μl of either ribonuclease T1 (1 mg ml^{-1} for 40 min at 37 °C) or pancreatic ribonuclease (1 mg ml^{-1} for 30 min at 37 °C) in 0.01 M Tris–HCl, *p*H 7.6, 0.001 M EDTA in the presence of 10 μg of tRNA carrier as described previously[12,20]. The origin of each autoradiograph is at the lower right. The first dimension (right to left) consists of high-voltage electrophoresis on cellulose acetate at *p*H 3.5, while the second consists of ascending RNA homochromatography on thin layers of DEAE-cellulose[20]. Fingerprints were exposed to Dupont Cronex 2 X-ray film at −70 °C in 14in. × 17in. Halsey–Radelin cassettes in the presence of two Dupont Lightning-plus intensifying screens. Fingerprints of 6 of the 11 PSTV isolates studied are shown. In each case, the ribonuclease T1 fingerprint is placed vertically above the pancreatic ribonuclease fingerprint; *a* and *g*, severe isolate, PS; *b* and *h*, severe isolate, SE; *c* and *i*, intermediate isolate, PD; *d* and *j*, mild isolate, PM; *e* and *k*, mild isolate, 6; *f* and *l*, mild isolate, 12. For an account of the source of each of these isolates, see the footnotes to Table 1. The numbered arrows in panels *a*, *d* and *g* indicate spots which are diagnostic for one or another strain of PSTV as discussed in the text and summarised in Table 1.

severe disease symptoms[1,4]. The molecular mechanisms by which viroids replicate and interact with their hosts are not yet understood. In its most severe form, the disease[5,6] caused by potato spindle tuber viroid (PSTV) causes general stunting of potato plant growth, deformity of the upper foliage, and production of disfigured potatoes[5]. Mild strains of PSTV which produce barely detectable symptoms have also been isolated[7]. Furthermore, plants infected by mild strains are somehow protected from developing symptoms following subsequent inoculation with severe strains[8,9]. This biological protection phenomenon has been called cross-protection[7]. As the only known component of viroids is RNA, mild and severe strains must differ in nucleotide sequence. The study described here aimed to determine the extent of this difference.

Four independently maintained severe, one intermediate, and six mild isolates of PSTV were propagated in Rutgers tomato plants, extracted and purified as described elsewhere[10], labelled *in vitro* with ^{125}I (ref. 11), and subjected to two-dimensional RNA fingerprint analysis[11–13]. Because the fingerprints obtained showed evidence of variable quantities of host RNA contaminants (data not shown), the ^{125}I-labelled viroid preparations were repurified by electrophoresis in 5% polyacrylamide gels[11] and fingerprinted once again.

Ribonuclease T1 and pancreatic ribonuclease fingerprints of two severe, one intermediate, and three mild PSTV isolates are shown in Fig. 1. The other two severe and three mild isolates studied did not differ significantly from those illustrated. Visual inspection reveals that all six ribonuclease T1 fingerprints closely resemble each other, as do all six pancreatic ribonuclease fingerprints. However, a few differences are evident. The four spots found to be diagnostic for the various strains are indicated in Fig. 1 by numbered arrows. The four severe strains have spots 1, 2 and 4 but lack spot 3 (Fig. 1 *a*, *b*, *g*, *h*). The intermediate strain has spots 2 and 4 but lacks spots 1 and 3 (Fig. 1 *c*, *i*). The six mild strains have spot 3 but lack spots 1, 2 and 4 (Fig. 1 *d*, *e*, *f*, *j*, *k*, *l*).

Secondary enzymatic digestion of all oligonucleotides from the fingerprints shown in Fig. 1 *a*, *d*, *g*, *j* followed by identification of cleavage products (as described in refs 12, 13) revealed only two further differences between the mild and severe strains of PSTV. The pancreatic ribonuclease fingerprint of mild PSTV contained the hexamer AAGGAC and the tetramer GAGC, neither of which can be detected by visual examination as all strains of PSTV analysed have two other hexanucleotides, GAGAAC and GGAAAC which co-migrate with AAGGAC; and AGGC which co-migrates with GAGC. As the sequence of spot 4 is AAAAAAGGAC, there is the attractive possibility that the new hexanucleotide AAGGAC found in mild strains is the result of the replacement of the fourth A-residue of spot 4 by a pyrimidine. These sequences were determined as described previously[12,14–17]. The intermediate strain (Fig. 1 *i*) lacks AAGGAC and contains GAGC.

Table 1 Oligonucleotide differences observed among severe, intermediate and mild strains of PSTV

Oligonucleotide description*	PSTV strain† Severe	Intermediate	Mild
spot 1	+‡	−	−
spot 2	+	+	−
spot 4	+	+	−
spot 3	−	−	+
AAGGAC	−	−	+
GAGC	−	+	+

* Spots 1, 2, 3 and 4 are those indicated by numbered arrows in Fig. 1.

† Eleven isolates of PSTV were selected for these studies. One of the four severe isolates (A) was obtained from the Schultz Collection of Potato Pathogens at Beltsville, Maryland. The other three severe isolates (PS, D and SE) were obtained over the course of 30 yr from naturally infected potatoes in field plots of the Cornell University Potato Breeding Program by Dr Karl H. Fernow. This was also the source of five of the six mild isolates (PM, 6, 7, 10 and 11). The sixth mild isolate (12) was recovered in autumn 1977 from a naturally infected potato plant at Riverhead, New York by Dr Fernow. The intermediate isolate, PD, was the gift of Dr T. O. Diener and was the one we used in previous RNA fingerprinting studies[11,13,16].

‡ + indicates the presence of the specified oligonucleotide in fingerprints of the PSTV strain in question. − indicates the absence of the specified oligonucleotide from fingerprints of the PSTV strain.

Thus, as shown in Table 1, while severe and mild strains of PSTV are uniquely characterised by the presence and absence of particular spots in their fingerprints, the intermediate strain has no such singular features. Detailed study of all data derived from oligonucleotide secondary analysis also permits us to estimate that mild and severe strains differ in 2–10 of their nucleotide residues (E.D. and H.D.R., unpublished observations).

These results demonstrate that the marked contrast between severe and mild symptoms must be explained on the basis of relatively few specific nucleotide sequence differences. As noted above, one of the sequence differences seems to occur at the fourth residue of the decanucleotide AAAAAAGGAC (spot 4: see Fig. 1 and Table 1). This can be identified as position 120 within the sequence of an intermediate strain[10] of PSTV (refs 18, 19). Further sequence analysis will be required before the exact positions of the other oligonucleotides associated with the mild-severe difference can be assigned. Such studies should also allow us to determine whether the residues important for the pathogenic properties are clustered in a small region of the viroid genome or are widely separated.

Although mild and severe strains of PSTV have very similar nucleotide sequences, additional studies indicate that such extensive sequence homology is not a prerequisite for cross-protection. Although comparative RNA fingerprint analysis of PSTV, citrus exocortis viroid (CEV) and chrysanthemum stunt viroid (CSV) have demonstrated that the sequences of these three viroids differ extensively from each other[11,13,16], biological studies have recently shown[9] that mild strains of PSTV cross-protect, not only against severe strains of PSTV, but also against CEV and CSV.

If cross-protection and disease symptom formation are determined by common features within a portion of the viroid genome, further studies should allow us to identify the regions and assess the influence of RNA sequence and structure on these biological properties.

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Selecting *Rhizobium* for acid, infertile soils of the tropics

AN increase in beef cattle production on the 350 million hectares of acid, infertile savannahs of tropical South America would have a major impact on world food production. Production in these regions is limited by the inadequate nutritional value of the native vegetation, and legume-based pastures are now being introduced[1] to raise the protein content of available forage. However, to obtain full benefit from biological nitrogen fixation through nodulated legumes it is necessary that the introduced legume form an effective symbiosis with *Rhizobium*. The low *p*H, limited availability of phosphorus, and very high levels of aluminium and manganese in these soils, are likely to affect nodulation and nitrogen fixation. It is therefore essential that strains of *Rhizobium* adapted to acid conditions are used. Alkali production by rhizobia from tropical legumes and the consequent rise in *p*H of test media has made the development of a simple test to select such strains difficult[2]. We report here that interference from alkali production can be eliminated by minor changes in the screening medium. This finding challenges the view that alkali production is a distinguishing characteristic with ecological significance[3], and is also a starting point for detailed study of the effects of acidity and related factors on growth of *Rhizobium*.

In an evaluation of 50 *Rhizobium* strains (from soils ranging from *p*H 4.5 to 9.0) isolated from nodules obtained from *Stylosanthes capitata*, *S. guianensis* and *S. hamata* we observed marked differences between strains in their ability to grow in two yeast–mannitol (YM) media differing in *p*H. Strains fell into one of three categories: 19 grew only in the standard YM medium of *p*H 6.8–7.0; 2 grew only in an acidified medium (adjusted to *p*H

4.5 with 0.1 M HCl); and 29 grew in both media. However, media which were acid initially had a final *p*H in the range 7 to 8 due to an alkalising effect which is claimed to be a differentiating characteristic of slow-growing tropical rhizobia[3]. Therefore, growth could indicate either genuine ability to multiply at low *p*H or capacity to progressively increase the *p*H of the medium to an adequate level for growth. A stable acid medium is needed that selects specifically for the former character as strains which grow as a result of influencing their environment during the test are unlikely to be successful in more highly buffered acid soils.

A range of media differing in composition and *p*H was evaluated as substrate for the growth of two strains of contrasting origins (acid soil, Llanos Orientales, Colombia, *p*H 4.5 compared with alkali soil, near Kingston, Jamaica, *p*H 8.5). The strain of alkali soil origin grew in media of initial *p*H 7 and *p*H 8.5 but not in a medium of *p*H 4.5. The strain of acid soil origin grew best in the medium of *p*H 4.5, made little growth at *p*H 7 and even less at *p*H 8.5. Growth of the acid soil origin strain was equally good irrespective of whether the medium was acidified using hydrochloric acid, sulphuric acid or citric acid. It also grew satisfactorily in media incorporating other acidity stresses, that is, low P (10 μM), high Al (50 μM), low Ca (50 μM), and high Mn (200 μM). Growth of ***Rhizobium*** in acidified media incorporating mannitol, the standard carbon source recommended for culturing rhizobia, raised the *p*H from 4.5 to approximately 7.5. However, in acidified media in which arabinose, or alternatively galactose, was substituted for mannitol, the *p*H decreased slightly (to 3.8) yet growth (turbidity/absorbance) was essentially the same as in media incorporating mannitol. Previous attempts to stabilise culture media at acid *p*H for growth of ***Rhizobium*** by using buffers have proved unsatisfactory[4]. Other reports concerning optimal *p*H for growth of slow-growing rhizobia[5,6] and utilisation of carbohydrates[7] have referred to initial *p*H values. Final *p*H values have been reported for growth in a range of carbon sources but from an initial *p*H of approximately 7 (ref. 8).

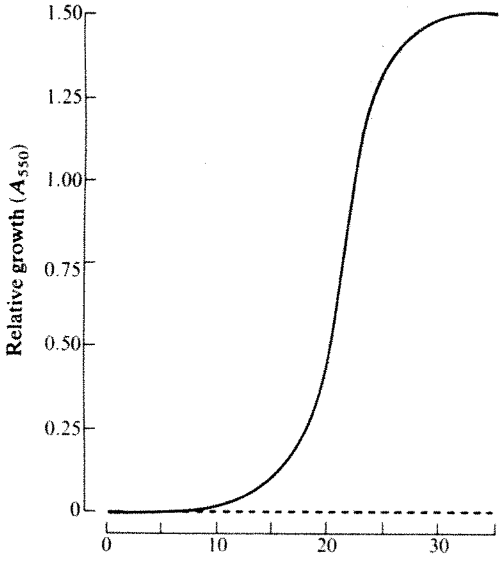

Fig. 1 Relative growth of *Rhizobium* strains CIAT 1460 (solid line), originating from an acid soil (*p*H 4.5), and CB 2126 (dashed line), from an alkali soil (*p*H 8.5), in a defined, acid medium (initial *p*H 4.2).

Table 1 Liquid medium for selecting acid-adapted strains of *Rhizobium*

Addition	Quantity added
KH_2PO_4	68 mg
K_2HPO_4	87 mg
$CaCl_2 \cdot 2H_2O$	39.4 mg
$MgSO_4 \cdot 7H_2O$	74 mg
Fe^{3+}Na EDTA	36.8 mg
$MnCl_2 \cdot 4H_2O$	0.49 mg
$ZnSO_4 \cdot 7H_2O$	0.29 mg
$CuCl_2 \cdot 2H_2O$	43 μg
$Na_2MoO_4 \cdot 2H_2O$	12 μg
$CoCl_2 \cdot 6H_2O$	1.2 μg
Sodium glutamate	220 mg
*Thiamine	100 μg
*Biotin	250 μg
Arabinose	10 g
Distilled water	to one litre

Acidified with 0.1 M HCl (before autoclaving).
* Filter sterilised and added after autoclaving the medium.

Growth of ***Rhizobium*** was quantified for a defined basal mineral salts liquid medium (Table 1) which was one of the media used previously. Cultures were incubated at 25–28 °C and aerated by shaking. Growth, both as optical absorbance at 550 μm and as viable cell number, and *p*H were determined over a period of 33 d in media which differed only in their carbon source (mannitol or arabinose). In both media, the acid soil origin strain CIAT 1460 showed exponential growth (Fig. 1), increasing from an inoculum of 7×10^3 to 1×10^9 viable cells per ml whereas the number of viable cells per ml of alkali soil origin strain CB 2126 diminished from 4×10^4 to 2×10^2. Growth of the acid soil strain in mannitol medium was associated with a substantial *p*H rise (Fig. 2); in arabinose medium, however, the *p*H declined from 4.2 to 3.9. Growth (viable cell number) of CIAT 1460 did not differ significantly in the two media.

The finding that some strains of ***Rhizobium*** grow much better at *p*H 4.5 than in neutral media is contrary to the commonly accepted view that ***Rhizobium*** has a *p*H growth optimum of ~7 (refs 9, 10). It also helps explain why attempts to isolate *Rhizobium* from nodules of *Stylosanthes* (especially *S. capitata*) growing in soils at *p*H 4.5–5.5 have been unsuccessful—the routine isolation medium has a *p*H 6.8–7.0. In addition it suggests that the standard practice of searching for acid stress tolerant ***Rhizobium*** in existing collections of strains that were originally isolated onto routine non-stress media may not be successful.

Now that this medium is available it will be possible to test the effects of characteristic acid-soil conditions, such as high aluminium content, on ***Rhizobium***. Comparison of strains in the early phase of their growth before a change in the *p*H of the

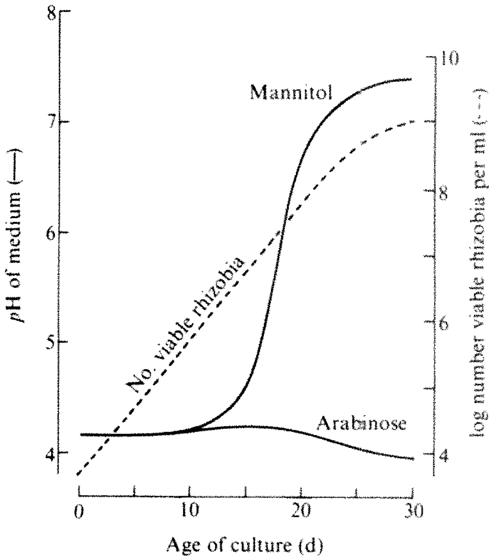

Fig. 2 The *p*H changes during culture of *Rhizobium* strain CIAT 1460 in defined liquid media containing either the standard carbohydrate source mannitol, or an alternative source, arabinose. Rhizobia multiplied at the same rate in both media from an initial viable cell number of 7×10^3 to 1×10^9 cells per ml.

medium can be detected[7] has the disadvantages that strain differences have not become fully pronounced and that the growth must be estimated by the extremely tedious viable count method. Availability of an acid medium in which the *p*H does not rise permits a simple test in which attainment of visual turbidity indicates ability to grow at low *p*H.

Finally, our demonstration that alkali production by slow-growing, tropical-type rhizobia is dependent on the carbohydrate of the culture medium reopens the question of whether these microorganisms actually produce alkali in the rhizosphere of their host and whether an ecological role should have been attributed to the process[3].

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Inactivation of restriction endonuclease *Bam*Nx after infection with phage ϕNR2

A HOST-CONTROLLED restriction–modification system[1-3] is a means of protection by a host against attacks of bacteriophages. Recently, coliphage T3 and T7 have been found to overcome[4-7] the host-controlled restriction–modification systems of their host strains *Escherichia coli* B and K, but details of the mechanism are not well understood at present. We have found that a *Bacillus subtilis* phage ϕNR2rH, a mutant of ϕNR2 (ref. 8), also has the ability to overcome the restriction–modification system of *B. amyloliquefaciens* strain N, and detected a *Bam*Nx inhibitor in cells infected with ϕNR2rH.

Shibata and Ando found two different restriction endonucleases[9-11], *Bam*NI (*Bam*NI cleaved DNA in the same nucleotide sequence as *Bam*HI (refs 12, 13)) and *Bam*Nx in the strain N. *Bam*Nx is a typical type II restriction endonuclease. The phage ϕNR2 and its host *B. subtilis* PS9 (derivative of *B. subtilis* Marburg strain sensitive to phage SP10 and ϕNR2) were isolated by Yajima *et al.*[8,14]. Since ϕNR2 DNA is completely insensitive to *Bam*NI, the phage ϕNR2 can form plaques normally on both PS9 and *B. amyloliquefaciens* strain H. ϕNR2 (grown on PS9) however, forms plaques on strain N at a frequency of about 4×10^{-4}. Initially we thought that the phage ϕNR2 was restricted by *Bam*Nx of strain N and that the survived phages would be modified. One clone, isolated from the plaques on strain N, was no longer restricted by this strain. Nevertheless, the DNA, prepared from phage particles grown on strain N, was still cleaved by *Bam*Nx (Fig. 1). These results suggested that this mutant has the ability to overcome the host-controlled restriction–modification system of strain N. The mutant was designated as ϕNR2rH because it formed rapid lysis-type plaques on strain H. ϕNR2rH was inactivated by anti-ϕNR2 serum, and its DNA gave the same *Eco*RI cleavage pattern as that of ϕNR2 DNA (data not shown), indicating that ϕNR2rH is a spontaneous mutant of ϕNR2.

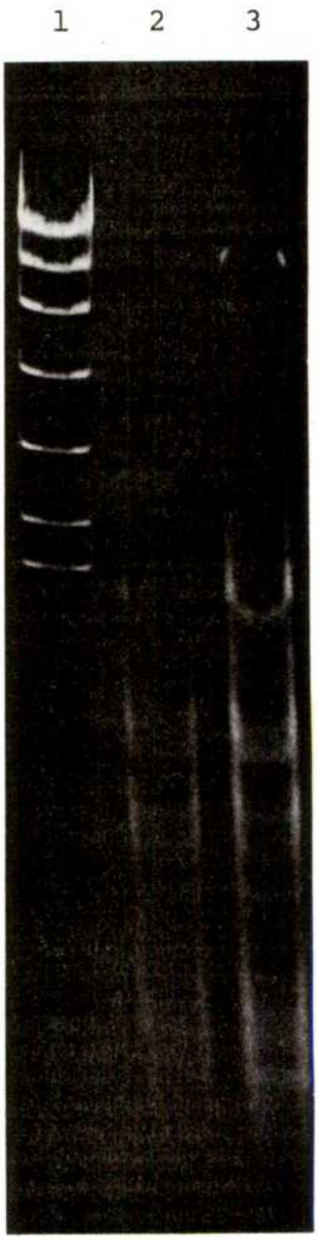

Fig. 1 Cleavage of DNAs of ϕNR2 (grown on PS9) and ϕNR2rH (grown on strain N) with endonuclease *Bam*Nx. Each DNA (1 μg) was reacted with *Bam*Nx in 20 μl of 20 mM Tris-HCl buffer (*p*H 7.5) containing 0.2 mM EDTA, 15 mM $MgCl_2$ and 5 mM 2-mercaptoethanol at 37 °C for 60 min. Cleaved DNAs were adjusted to 8% glycerol and 0.02% bromophenol blue and heated for 2 min at 60 °C. The samples were applied to 0.8% Agarose (Seakem) slab gel (15 × 15 × 0.3 cm). Electrophoresis was carried out at room temperature for 2.5 h at a constant current of 90 mA. The gel was stained with ethidium bromide and photographed with short-wave ultraviolet light. Löening's buffer[15] (36 mM Tris-HCl, *p*H 7.7, 30 mM NaH_2PO_4, 1 mM EDTA) was used for electrophoresis and staining[16]. 1, *Bam*NI fragments of *B. subtilis*-phage ρ11C3a DNA as standard markers (see Fig. 3 in detail); 2, ϕNR2 (grown on PS9) DNA cleaved with *Bam*Nx; 3, ϕNR2rH (grown on strain N) DNA cleaved with *Bam*Nx.

ϕNR2rH was not restricted by strain N, whereas its DNA was cleaved by *Bam*Nx from strain N, *in vitro*. We therefore examined whether or not the activity of *Bam*Nx still remains in N cells infected with ϕNR2rH. Crude extract was prepared from phage-infected cells by the following procedure . Cells of strain N were infected with ϕNR2rH at a multiplicity of five when the number of cells became approximately 1.5×10^8 ml^{-1} in L-broth at 37 °C with shaking. The infected cells were collected 15 min after infection and stored at −20 °C. These cells were then thawed, suspended in 20 mM Tris-HCl buffer (*p*H 7.5) containing 5 mM MgCl$_2$, 0.2 mM EDTA and 5 mM 2-mercaptoethanol, and sonicated for 15 min in an ice-water bath. After removal of the cell debris by centrifugation at 80,000*g* for 90 min at 4 °C, 5% streptomycin sulphate solution was added gradually, to give a final concentration of 1%. The suspension was centrifuged and the supernatant was dialysed at 4 °C overnight against the 20 mM Tris-HCl buffer described above. The precipitate which appeared during dialysis was removed by low-speed centrifugation. The final supernatant was the crude extract used in this study. Crude extracts were also prepared from ϕNR2-infected and uninfected N cells, as was the ϕNR2rH-infected cell extract. We could detect *Bam*Nx activity in the extracts from ϕNR2-infected and uninfected cells but not in the extract from ϕNR2rH-infected cells (data not shown). We concluded, therefore, that ϕNR2rH infection caused the inactivation of *Bam*Nx.

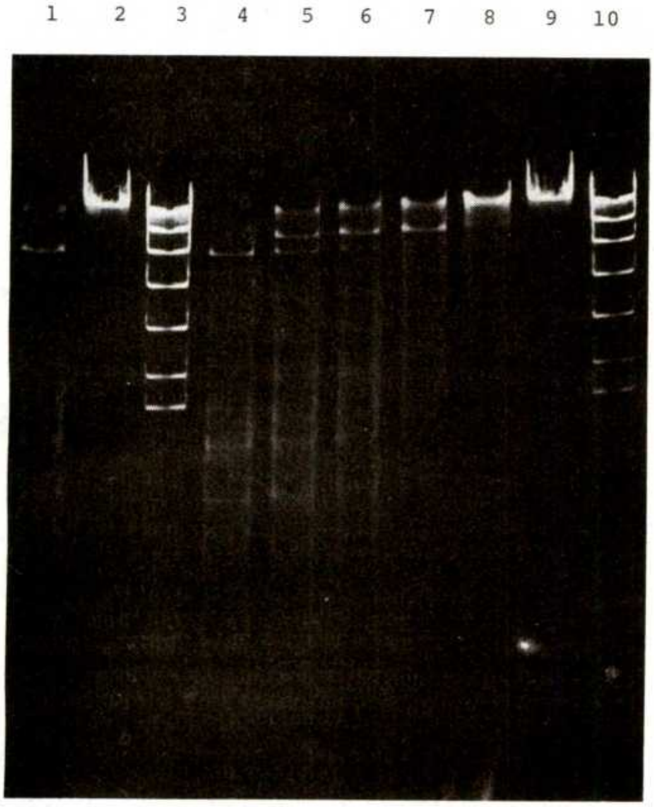

Fig. 2 Detection of *Bam*Nx inhibitor in the crude extract from ϕNR2rH-infected N cells using ϕNR2rH DNA as the substrate of *Bam*Nx. Purified *Bam*Nx was preincubated with various amounts of the crude extract, prepared as described in the text, in 50 μl of 20 mM Tris-HCl buffer (*p*H 7.5) containing 0.2 mM EDTA, 15 mM MgCl$_2$, 5 mM 2-mercaptoethanol at 37 °C for 15 min. After this treatment, 2.5 μg of ϕNR2rH DNA extracted from purified phage particles grown on strain N were added, incubated for 60 min at 37 °C and 30 μl of each sample were applied to electrophoresis for the analysis of the remaining *Bam*Nx activity. Electrophoresis was carried out as described in Fig. 1. *Bam*NI fragments of *B. subtilis*-phage ρ11C3a DNA were used as standard markers (see Fig. 3 in detail). 1, Remaining activity of *Bam*Nx after the preincubation with 25 μl of the crude extract (about 7 mg protein per ml) from uninfected N cells; 2, untreated ϕNR2rH DNA; 3, *Bam*NI fragments of ρ11C3a DNA; 4–8, inactivation of *Bam*Nx by the preincubation with the crude extract from ϕNR2rH-infected N cells. Purified *Bam*Nx was preincubated with 0 μl (4), 1 μl (5), 3 μl (6), 10 μl (7) and 25 μl (8) of the crude extract from ϕNR2rH-infected N cells (about 8 mg protein per ml). 9, untreated ϕNR2rH DNA; 10, *Bam*NI fragments of ρ11C3a DNA.

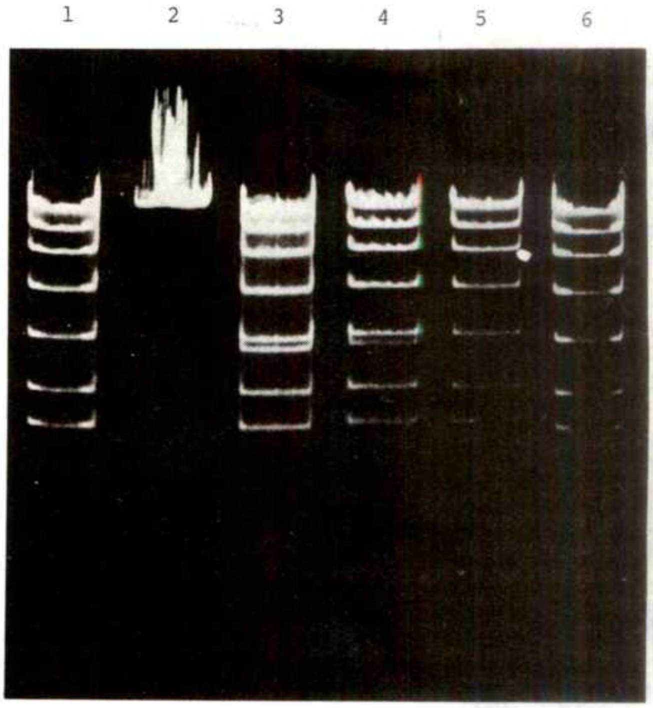

Fig. 3 Detection of *Bam*Nx inhibitor using ρ11C3a DNA as the substrate of *Bam*Nx. The same crude extracts as described in Fig. 2 were used. *B. subtilis*-phage ρ11C3a DNA generates 10 fragments by *Bam*NI (or *Bam*HI) cleavage, and 2 fragments by *Bam*Nx cleavage (Kawamura and Ito, in preparation). *Bam*NI fragments were designated *Bam*NI A–J in order of decreasing size. *Bam*Nx cleaves *Bam*NI A fragment and a new DNA band appears between the *Bam*NI E and F fragments by Agarose gel electrophoresis after cleavage with both *Bam*NI and *Bam*Nx. It is thus possible to determine the activity of *Bam*NI and/or *Bam*Nx using this DNA as substrate. The preincubation of purified *Bam*Nx with the crude extract was performed in the same conditions as described in Fig. 2. Electrophoresis was carried out as described in Fig. 1. 1, *Bam*NI fragments of ρ11C3a DNA; 2, untreated ρ11C3a DNA; 3, fragments of ρ11C3a DNA generated by purified *Bam*NI and purified *Bam*Nx; 4, remaining activity of *Bam*Nx after the preincubation with 25 μl of the crude extract from uninfected N cells; 5, inactivation of *Bam*Nx by the preincubation with 25 μl of the crude extract from ϕNR2rH-infected N cells. 4 and 5 also show that *Bam*NI activity still remains in ϕNR2rH-infected N cells. 6, *Bam*NI fragments of ρ11C3a DNA.

*Bam*Nx, purified according to the method described by Shibata and Ando[11], was preincubated for 15 min at 37 °C with various amounts of the crude extract, and the remaining activity of *Bam*Nx was determined by Agarose gel electrophoresis. *Bam*Nx activity was seriously inhibited by the preincubation with crude extract prepared from ϕNR2rH-infected N cells (Figs 2 and 3). This suggested that the *Bam*Nx inhibitor appears after the ϕNR2rH infection, and that this *Bam*Nx inhibitor is responsible for overcoming the host-controlled restriction–modification system of strain N.

In order to reveal the molecular function of the *Bam*Nx inhibitor in detail, it will be necessary to purify it, but our preliminary data indicate that it is proteinous and inhibits *Bam*Nx specifically.

Phage ϕNR2rH, like T3 and T7, has the function of overcoming the host-controlled restriction–modification system. The restriction endonucleases overcome by T3 and T7 belong to type I. Phage ϕNR2rH may be the first example of a shape overcoming the type II restriction endonuclease of its host strain. A similar phenomenon might be found in further studies, and if so, it would have biological importance in host–parasite interactions.

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Dependence of protein synthesis on RNA synthesis during the early hours of germination of wheat embryos

WHEAT EMBRYO germination is characterised by an increase in fresh weight during the first 40 min of imbibition, followed by a lag period of 5 h and finally, a phase of cell elongation during which fresh weight increases at a sustained rate[1]. An essential step in seed germination is *de novo* synthesis of proteins, but for the first 40 min it is mediated by preformed mRNA transcribed during embryogenesis[2]. Previous reports have shown that although there is a 2.5–3-fold increase in the relative rate of protein synthesis during the lag period (40 min–5.5 h)[3], there is no appreciable change in the rate of RNA synthesis[4-7]. With the recently developed sensitive assays for ribonucleoside triphosphates[8,9], the rate of RNA synthesis and its mode of regulation in early germination of wheat embryos was re-examined[10]. In contrast to previous findings[4-7], our results clearly show a two- to threefold increase in the rate of RNA synthesis during the lag period of wheat embryo germination. This increase in RNA synthesis could conceivably account for the threefold increase in the rate of protein synthesis[4] and raises the possibility that protein synthesis may depend on RNA synthesis after the first 40 min of germination. We describe here experiments undertaken in wheat (*Triticum aestivum* L.) embryos to study the effect of inhibition of RNA synthesis on the rate of protein synthesis. Our data suggest that protein synthesis depends on RNA synthesis, and that the substrate level of mRNA is not a rate-limiting determinant for protein synthesis during the early hours of germination.

Fig. 1 Isolated wheat embryos (100 μg) were germinated for 3 h in the presence or absence of α-amanitin. Ribosomes were isolated as described by Spiegel *et al.*[5]. Four A_{260} units of ribosomes were separated on a 17–42% linear sucrose gradient with a 0.5 ml sucrose (1.8 M) pad in a Beckman SW 50.1 rotor by centrifuging at 49,000 r.p.m. for 40 min. *a*, control; *b*, treated with 2 $\mu g\,ml^{-1}$ of α-amanitin; *c*, treated with 10 $\mu g\,ml^{-1}$ of α-amanitin.

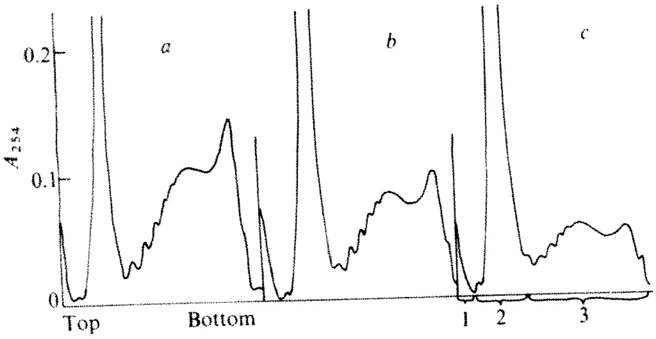

Table 1 Effect of α-amanitin on RNA(*a*) and protein (*b*) synthesis

a

α-Amanitin added ($\mu g\,ml^{-1}$)	$[5\text{-}^3\text{H}]$uridine incorporation c.p.m.	%	Total mRNA c.p.m. per μg	%	Ribosome-associated mRNA c.p.m. per μg	%
0	27,600	100	13,600	100	19,500	100
2	17,300	63	10,600	78	17,800	91
4	14,100	51	–	–	–	–
10	9,000	33	7,300	54	12,700	65

b

α-Amanitin added ($\mu g\,ml^{-1}$)	$[4,5\text{-}^3\text{H}]$leucine incorporation c.p.m.	%	Polysome content %	Active ribosome c.p.m. per 0·1 A_{260} units	%
0	98,200	100	100	31,100	100
2	58,900	60	79	25,500	82
4	42,200	43	—	—	—
10	20,600	21	54	17,400	56

a, Isolated wheat embryos were germinated as described previously for 3 h in the presence or absence of α-amanitin[10]. After 3 h of germination, 10 μCi of $[5\text{-}^3\text{H}]$uridine was used to label 50 mg of isolated wheat embryos for 30 min. The 5% trichloroacetic acid-insoluble material was prepared as described previously[10]. 50 mg of germinated wheat embryos were ground with 0.4 ml of high salt buffer (1 M KCl, 0.1 M Tris-HCl (*p*H 7.6) and 10 mM EDTA) with a mortar and pestle. 2 ml of 1% SDS buffer (5 mM EDTA, 10 mM Tris-HCl (*p*H 7.6) and 1% SDS) was then added to the extract, mixed well and extracted twice with 3 ml of water-saturated chloroform–phenol (1 : 1, v/v). The aqueous phase was adjusted to 0.2 M ammonium acetate and the RNA was precipitated by 2 volumes absolute ethyl alcohol (18 h, −20 °C). The precipitate was washed 3 times with 2 M LiCl, twice with 70% aqueous ethyl alcohol, dried and dissolved in 2 ml of sterilised water. The total RNA (6.25 μg) was translated in a wheatgerm extract S30 system[15]. Ribosomes were obtained from 100 mg of isolated wheat embryos germinated for 3 h as described by Spiegel *et al.*[5]. The ribosomal pellet was suspended in 2 ml of 1% SDS buffer and then mixed with 1 ml of regular buffer (5 mM EDTA and 10 mM Tris-HCl, *p*H 7.6). 400 μl of high salt buffer were added to the ribosome suspension. The resulting precipitate was removed by centrifugation. 3 ml of water-saturated chloroform–phenol were used to extract the aqueous phase. The RNA was prepared as described before. The wheatgerm extract S30 was used to translate 5 μg of ribosome-associated mRNA. *b*, After 3 h of germination, the embryos were labelled with 20 μCi of L-$[4,5\text{-}^3\text{H(N)}]$leucine for 30 min. The 5% trichloroacetic acid-homogenate was prepared as described previously[10], except that it had been incubated at 90 °C for 15 min. The polysome content was obtained from Fig. 1 by measuring the area of the polysome region from absorbance compared with the fraction plot following sucrose gradient centrifugation. Ribosomal fractions were prepared as described above. Excess wheatgerm extract S100 (5 μl) was used for the completion of nascent peptidyl chains present in 0.16 A_{260} units of ribosome in the presence of 8 μM pactamycin.

After 3 h of germination, concomitant decreases in RNA and protein synthesis were observed in isolated wheat embryos treated with α-amanitin (Table 1, column 3). Measurements of the fractionation of ribosomes isolated from α-amanitin-treated samples on a 17–42% sucrose gradient revealed a decrease in polysomes and an increase in monosomes (Fig. 1). With α-amanitin at 2 $\mu g\,ml^{-1}$ and 10 $\mu g\,ml^{-1}$, the polysome content was depressed by 21% and 46%, respectively (Table 1*b*, column 4). Active ribosomes were quantified by allowing the nascent peptidyl chains of active ribosomes to complete polypeptide

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synthesis in the presence of the initiation inhibitor, pactamycin. The treatment of α-amanitin at $2\ \mu g\ ml^{-1}$ and $10\ \mu g\ ml^{-1}$ caused a decrease in the amount of active ribosomes by 18% and 44%, respectively (Table 1*b*, column 5). The possibility of a direct effect by α-amanitin on protein synthesis was eliminated by an *in vitro* study with a cell-free wheatgerm translation system (data not shown).

α-Amanitin probably inhibits the synthesis of mRNA which in turn may affect either the substrate level of mRNA or the cellular content of an essential factor(s) required for protein synthesis. After 3 h of germination, the cellular level of total mRNA was 22% and 46% lower than the control sample when treated with α-amanitin at $2\ \mu g\ ml^{-1}$ and $10\ \mu g\ ml^{-1}$, respectively (Table 1*a*, column 5). The amount of mRNA actually associated with ribosome in α-amanitin-treated samples was decreased to a lesser extent. When $2\ \mu g\ ml^{-1}$ of α-amanitin was used in the incubation, the amount of ribosome-associated mRNA decreased by only 9% (Table 1, column 7). Such a small decrease in the ribosome-associated mRNA cannot fully account for the 40% inhibition of protein synthesis (Table 1*b*, column 3) and the 20% depression of polysome content (Table 1*b*, column 4). Therefore, the substrate level of mRNA does not seem to be the rate-limiting determinant for protein synthesis.

No obvious changes in the polysome profile were observed following treatment with α-amanitin (Fig. 1). This observation would suggest that normal loading of ribosomes on the mRNA can proceed once the mRNA is 'activated' for protein synthesis. A reduced initiation efficiency in protein synthesis has been observed in other cell systems in resting compared with proliferating cultures[11,12]. It is possible that the formation of the first peptidyl bond is required by the essential factor(s), as the first dipeptide synthesis is a unique step in protein synthesis[13]. It has also been reported that protein synthesis is not regulated by the supply of mRNA in oocytes of *Xenopus laevis*[14].

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MHC matching shows that at least two T-cell subsets determine resistance to HSV

DISTINCT T-cell subsets recognise antigens in association with different major histocompatibility complex (MHC) products, and this provides a means of identifying the subsets which operate in protective immunity against pathogens. Thus, cytotoxic T cells (T_C) recognise antigen in association with products of the K and D regions of the H–2 complex of the mouse; and because protection against lymphocytic choriomeningitis[1], ectromelia[2] and influenza[3] requires compatibility at K or D in adoptive transfer experiments, it follows that the subset mediates protective immunity against these viruses. I-region compatibility requirements have not hitherto been observed in viral infections, but are necessary for protection against the intracellular parasite *Listeria monocytogenes*. This suggests that T helper cells (T_H) and/or the lymphocytes responsible for delayed hypersensitivity and lymphokine release may be involved[4] (these lymphocytes can provisionally be included within the T_H subset). Adoptive transfer studies in mice have clearly established a major role for T lymphocytes in recovery from infection with herpes simplex virus (HSV-1)[5–7]. The T-lymphocyte subclass or classes responsible for this protection are not known. *In vitro* cytotoxic T cells (T_C)[8], and their primed precursors[9] can be obtained from HSV-1 infected mice. The present study indicates that immune protection against HSV-1 can be conferred by immune spleen cells possessing either I or K-D compatibility, and that long-lasting protection is conferred only by cells with I-region compatibility.

Table 1 H–2 compatibility required for immunological protection against HSV-1: adoptive transfer to AQR B mice

Donors (H–2 haplotype), no. and type of spleen cells transferred	Survival at 14 d	Survival at 60 d
AQR (qkkddd)		
5×10^7 normal	1/9	1/9
5×10^7 immune	11/11	11/11
$1–1.5\times10^7$ immune	10/12	10/12
5×10^6 immune	0/4	–
B10.BR (kkkkkk)		
5×10^7 normal	1/9	1/9
5×10^7 immune	9/9	9/9
$1–1.5\times10^7$ immune	3/12	3/12
B10.T(6R) (qqqqqd)		
5×10^7 normal	0/8	–
5×10^7 immune	7/10	2/10
$1–1.5\times10^7$ immune	4/11	0/11
B10.BR+B10.T(6R)		
$1.5\times10^7+5\times10^6$ immune	5/11	5/11
C57BL/6(bbbbbb)		
5×10^7 immune	2/10	2/10
$1–1.5\times10^7$ immune	0/8	–

Donor strains of mice (Table 1) were immunised initially by intraperitoneal injection of 10^5 plaque-forming units (PFU) of Kruger strain HSV-1, boosted with 10^7 PFU after 1 week and 10^6 PFU after 2 weeks (virus was grown on cell strain BHK-13, and stored at −70 °C at a concentration of 10^9 PFU ml^{-1}). After 10–14 d, pooled sera from each strain of donor mice consistently showed antibody in dilutions of 4×10^{-4} by a solid phase radioimmunoassay. At this time teased spleen cells were obtained aseptically, washed three times with a modified Eagle's medium and injected into recipients in the numbers indicated in Table 1. Recipients were 12–16-week-old AQR mice which had been adult thymectomised at 4–6 weeks of age, and at 8–10 weeks had been irradiated (750–800 R) and reconstituted with $1–2\times10^7$ AQR bone marrow cells following anti-Thy-1.2 and complement treatment ('B' mice).

Two days after spleen cell transfer, 2×10^6 PFU of HSV-1 were injected intraperitoneally; this was a quantity of virus found to be lethal for AQR B mice, which were themselves 100-fold more susceptible to the virus than normal mice of the same strain. Times of death were recorded and fell into two distinct groups. Mice dying less than 14 d after HSV-1 developed a progressive paralysis and findings typical of HSV

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encephalitis[6]. A second group of mice died 30–60 d after HSV-1. Those mice surviving beyond 60 d were considered to have a lasting immunity.

As Table 1 indicates 5×10^7 syngeneic immune cells (AQR) and 5×10^7 immune cells with I-region compatibility (B10.BR) conferred a long-lasting protection; $1\text{–}1.5\times10^7$ AQR immune spleen cells were also protective, but this number of B10.BR cells were protective in only 3/12 mice; 5×10^7 immune spleen cells with K-D compatibility, B10.T(6R), were protective initially in 7/10 mice but only 2/10 mice survived 60 d; $1\text{–}1.5\times10^7$ B10.T(6R) immune cells were initially protective in 4/11 instances, but long-lasting immunity was not achieved in any mouse. An attempt at enhancing protection by combining immune cells from two strains of mice, one with I-region compatibility (1.5×10^7 B10.BR) and the other K-D compatibility (5×10^6 B10.T(6R)) was successful in only 5/11 mice; this effect was long lasting, but in numbers not significantly different from that obtained with B10.BR cells alone. Spleen cells from non-immunised donors and from an immunised strain without H-2 compatibility (C57BL/6) failed to provide protection. By contrast, 5×10^7 of C57BL/6 immune spleen cells provided 100% protection in 26 acutely irradiated, HSV-infected B6 mice (350–450 R); spleen cells from non-immunised C57BL/6 mice failed to protect (18/21 deaths in less than 14 d).

These results demonstrate the need for H-2 compatibility in immune protection against HSV-1 in adoptive transfer studies in mice. Immune spleen cells from a mouse strain with known high resistance to herpes infection C57BL/6 (ref. 10), and demonstrated capacity to protect acutely irradiated HSV-1-infected syngeneic mice, fail to protect in non-H-2-compatible recipients. The protection conferred by H-2-compatible immune spleen cells is mediated by T cells, as we have confirmed previous work[7] showing that treatment of the transferred cells with anti-Thy-1.2 and complement abolished their protective effect (data not shown).

T-cell recognition of both I and K-D coded self-markers seems to be important in immune protection against HSV-1 in mice. I-region compatibility confers a long-lasting protection, whereas K-D compatibility confers only a temporary protection in most animals. These findings are consistent with a role for both T_H lymphocytes and T_C lymphocytes in the immune response to this virus, and complete protection is provided by T_H lymphocytes alone in sufficient numbers. It might be argued that T_C lymphocytes may also have played a part in long-term protection, through differentiation of precursors in the thymus of the K-D-imcompatible host; however, this seems unlikely because such precursors are not known to occur among spleen cells. This is therefore the first demonstration that T_H cells may have a greater protective capacity than T_C in a virus infection. It should be emphasised that this study differs from the previous short-term studies using recoverable virus as an end point, which have demonstrated a need for K-D compatibility only. T_H cells may prove important in recovery from these infections, as *in vitro* experiments suggest[11]. The interpretation of our long-term effects are open to question because of the problem of reaction to the alloantigens, but the significant point is that long-term protection was in fact obtained. We think it unlikely that the long-term protection can be attributed simply to an allogeneic effect, not only because of its duration, but also because it was obtained with K-D-incompatible rather than I-incompatible cells.

T_H could be providing protection in several ways, including helping T_C precursors or B cells, the elaboration of lymphokines leading to the recruitment of other cells, and the direct activation of macrophages[4]. The importance of mature macrophages as effector cells in recovery from HSV-1 infections has been stressed previously[7,12,13]. Help for B cells in the production of anti-viral antibody is perhaps the most straightforward possibility; the fact that anti-Thy-1 treatment abolishes protection does not argue against this possibility, for such treatment would inactivate the helper cells known to participate in the secondary antibody response to HSV and other viruses[14]. The failure to achieve a protection comparable to that with syngeneic cells by a combination of two cell sources, one with I-region and the other with K-D-region compatibility suggests that compatibility may be required between T_H and T_C to achieve complete protection with low numbers of transferred immune cells.

Immune spleen cells recognising K-D-coded self-antigens only are also initially protective, strongly suggesting that T_C cells play a part in the immune response to herpes *in vivo* as well as *in vitro*[9]. The failure of such adoptively transferred mice to survive indefinitely may be due to eventual loss of specifically immune short-lived lymphocytes, although other mechanisms such as a graft-versus-host response could not be excluded in the present study.

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Evidence for a specific B27-associated cell surface marker on lymphocytes of patients with ankylosing spondylitis

THE strong association of HLA-B27 with ankylosing spondylitis (AS) as well as an increased frequency of B27 in other seronegative arthropathies is well documented[1–4]. Nevertheless, the significance and the molecular basis of this remarkable association is still unknown. Ebringer *et al.*[5–7] reported that *Klebsiella pneumoniae* was present in the faeces of patients with AS more frequently than in those of healthy controls, and that there seemed to be some cross-reactivity between HLA-B27-positive lymphocytes and *Klebsiella* antigen. It is possible, therefore, that an infectious agent, probably an enteric organism, is involved in the pathogenesis of AS. We have therefore decided to examine the *in vitro* proliferative responses of peripheral blood lymphocytes to *Klebsiella* and to several enteric organisms. To examine the possibility of cross-reactivity between B27 and *Klebsiella* antigen, we raised antisera in rabbits against various isolates of *Klebsiella* and tested the cytotoxicity of these antisera on lymphocytes of AS patients and of normal controls. The results presented here strongly suggest

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Table 1 Comparison of means of $\log_{10}$ transformed stimulation indices to various antigens of B27$^+$AS$^+$ patients compared with B27$^-$AS$^-$ and B27$^+$AS$^-$ normal controls

Antigen	Mean ± s.e.m. of $\log_{10}$ transformed stimulation indices			*t* values		
	B27$^+$AS$^+$ (*a*)	B27$^+$AS$^-$ (*b*)	B27$^-$AS$^-$ (*c*)	*a* v *b*	*a* v *c*	*b* v *c*
Klebsiella 411	0.22 (±0.02)	0.54 (±0.02)	0.64 (±0.02)	3.2*	4.8†	0.9
Klebsiella 427	0.12 (±0.02)	0.58 (±0.02)	0.56 (±0.02)	5†	5.5†	0.2
Klebsiella 433	0.13 (±0.02)	0.47 (±0.04)	0.58 (±0.02)	3.4*	5.1†	0.01
Klebsiella 462	0.05 (±0.01)	0.38 (±0.04)	0.40 (±0.03)	3.4*	4.8†	0.11
Klebsiella 468	0.08 (±0.01)	0.46 (±0.02)	0.37 (±0.02)	4.2†	4.1†	0.9
Staphylococcus	0.60 (±0.01)	0.75 (±0.17)	0.79 (±0.04)	1.0	1.7	0.5
Streptococcus	0.96 (±0.03)	0.87 (±0.12)	1.0 (±0.02)	0.6	1.2	0.4
Shigella	0.88 (±0.03)	0.89 (±0.07)	0.94 (±0.04)	0.2	0.7	0.5
Yersinia	0.69 (±0.02)	0.7 (±0.1)	0.72 (±0.01)	0.03	0.3	0.2
Phytohaemagglutinin	1.9 (±0.02)	1.8 (±0.03)	1.8 (±0.03)	1.23	1.1	1.0

The distribution of the stimulation indices for each antigen and each group of individuals was normalised by $\log_{10}$ transformation and the means of the $\log_{10}$ transformed values compared using the Student's *t* test (* $P<0.01$, † $P<0.001$). Peripheral blood lymphocytes were obtained from groups of B27-positive patients with ankylosing spondylitis (B27$^+$AS$^+$, $n=25$), B27-negative normal controls (B27$^-$AS$^-$, $n=37$) and B27-positive normal controls (B27$^+$AS$^-$, $n=13$). The cells were separated from heparinised blood by centrifugation in Ficoll–Paque (Pharmacia) for 25 min at 580*g*. The leukocyte band was removed and washed with RPMI 1640 (Flow) medium containing 2 ml heparin (1,000) units ml^{-1}), 10 ml 1M HEPES (Sigma) buffer, 5 ml L-glutamine (200 mM) and 2 ml penicillin–streptomycin (5,000 IU, 5,000 μg ml^{-1}; Flow) per 500 ml of RPMI medium. The leukocytes were suspended in RPMI medium containing 10% human AB serum and centrifuged for a further 10 min at 180*g*. The cells were then resuspended in RPMI medium containing 20% AB serum and adjusted to a cell concentration of 10^6 mononuclear cells per ml of medium. Bacterial suspensions were adjusted to either 10^6 (*Klebsiella*) or 10^9 (*Staphylococcus*, *Streptococcus*, *Yersinia* and *Shigella*) organisms per ml RPMI containing 20% AB serum. Lymphocytes (5×10^4 in 50 μl) were cultured in round-bottomed Linbro culture plates (Linbro) with 50 μl of the bacterial suspension (5×10^4 to 5×10^6 organisms) and 50 μl of RPMI containing 20% AB serum. All cultures were carried out in triplicate. In addition to the bacterial suspensions, the lymphocytes of each individual were cultured with phytohaemagglutinin (10 μg per well). The lymphocyte cultures were incubated at 37 °C for 6 d. On day 5, 1 μl of ^{3}H-thymidine (1 μCi) was added to each well and the cells collected about 24 h later, with a Skatron (Lierbyen) multiple cell culture collector. The radioactivity of the cells was measured in a liquid scintillation counter (Packard, TRI-CARB, model 2002). Results are expressed as stimulation indices: c.p.m. with antigen per c.p.m. without antigen.

Table 2 Antiserum to *Klebsiella* sp. (427) lyses lymphocytes of B27$^+$AS$^+$ patients but not of B27$^+$AS$^-$ or B27$^-$AS$^-$ healthy controls

Serum	Absorption	Target lymphocytes			
		B27$^+$AS$^+$	B27$^-$AS$^+$	B27$^+$AS$^-$	B27$^-$AS$^-$
Anti-B27	0	44	0	67	0
Anti-*Klebsiella* 427	0	69	1	11	10
Anti-*Klebsiella* 427	B27$^+$AS$^+$	13	nt	2	7
Anti-*Klebsiella* 427	B27$^+$AS$^-$	73	nt	10	3
Anti-*Klebsiella* 427	B27$^-$AS$^-$	64	nt	13	2
Anti-*Klebsiella* 468	0	11	12	6	13
Anti-*Klebsiella* 462	0	2	6	8	0
Anti-*Klebsiella* 433	0	8	7	12	3
Anti-*S typhimurium*	0	5	2	0	5
Anti-*S. typhi*	0	3	0	0	2
Anti-*Streptococcus* Group A	0	19	18	3	7
Anti-*Shig. dysenteriae*	0	2	0	4	5

Antisera to formalinised suspensions of various bacteria were tested on Ficoll-sedimented peripheral blood lymphocytes (see legend to Table 1) of B27$^+$AS$^+$ and B27$^-$AS$^+$ patients as well as B27$^+$AS$^-$ and B27$^-$AS$^-$ unaffected individuals in a ^{51}Cr release assay. About 10×10^6 lymphocytes were incubated in 1 ml of RPMI 1640 (Flow) containing 10% human AB serum and 100 μCi of ^{51}Cr (specific activity: 200–500 μCi per μg, Amersham) for 60 min at 37 °C. The cells were then washed twice with RPMI + 10% AB serum and resuspended at a concentration of 3×10^6 cells per ml. 100 μl of the ^{51}Cr-labelled cell suspension were added to 10×75-mm plastic tubes and incubated with 100 μl of antiserum at 24 °C for 30 min. Rabbit complement (100 μl) was then added to each tube and the incubation continued for a further 60 min. The cells were centrifuged at 2,000 r.p.m. for 15 min and 100 μl of the supernatant was counted in a Packard auto-gamma scintillation spectrometer. The amount of radioactivity in the samples was compared with radioactivity present in an equal volume of the supernatant from tubes containing cells in medium plus complement only, and with that present in tubes containing cells which were frozen and thawed three times. The results are expressed as the percentage of maximum ^{51}Cr released, which was calculated as follows:

$$^{51}\text{Cr realease \%} = 100\times\frac{\text{Radioactivity released by antiserum} - \text{radioactivity released in absence of antiserum}}{\text{Radioactivity released by frozen and thawed cells} - \text{radioactivity released in absence of antiserum}}.$$

The results shown are of a typical experiment on the cells of single individuals for each category. The antibacterial activity on the rabbit sera was demonstrated by the slide agglutination technique. Both the absorbed and unabsorbed antibacterial antisera were tested at a dilution of 1 : 8; anti-B27 antiserum was used undiluted. Anti-*Klebsiella* 427 antiserum was absorbed twice by mixing 100 μl of antiserum with 30×10^6 packed peripheral blood lymphocytes for 2 h at 24 °C The absorbed serum was centrifuged at 10,000 r.p.m for 30 min and diluted (1 · 8) with RPMI + 10% AB serum before testing.

that *Klebsiella* antigens cross-react with a gene product closely associated with HLA-B27 or with a *Klebsiella*-associated B27 antigen in AS patients.

As patients with AS have diminished T-cell responses to *Yersinia in vitro*[8], we wondered whether there was a similar impairment of *in vitro* T-cell proliferation in response to other bacterial antigens. Lymphocytes were obtained from B27-positive patients with definite ankylosing spondylitis (by Rome and New York criteria[9]) as well as from B27-positive and B27-negative healthy controls. Bacterial cultures were isolated from faecal samples (*Shigella flexner* and *Klebsiella*, isolate numbers 411, 427, 433, 462 and 468), a wound swab (*Staphylococcus* sp.) and a throat swab (Streptococcus sp.). *Yersinia enterocolitica* (NCTC no. 10598) was also used (American Type Culture Collection). Bacteria were killed by adding 0.25 ml of 40% formaldehyde to a 20-ml suspension of the organisms (10^9–10^{10} per ml) in nutrient broth. The formalin-killed bacteria were washed twice with sterile 0.9% NaCl and stored at −20 °C.

Table 3 Lymphocytotoxic activity of Anti-*Klebsiella* 427 antiserum for lymphocytes from AS patients and from normal controls

Diagnosis	B27 positive	No. positive/no. tested
Definite AS	Yes	21/23
Probable AS	Yes	3/3
Definite AS	No	0/3
Normal	Yes	0/13
Normal	No	0/22

Diagnosis was based on Rome and New York criteria.

The data in Table 1 show that B27-positive patients with AS as well as B27-positive and B27-negative healthy controls respond equally well to phytohaemagglutinin *in vitro*. Similarly, the responses of individuals, in all three groups, to *Staphylococcus*, *Streptococcus*, *Y. enterocolitica* and *Shig. flexner* are not significantly different. Commercially obtained preparations of *Salmonella tryphimurium* H specific and *S. typhi* H (Wellcome) failed to stimulate the lymphocytes of any of the individuals tested (data not shown). By contrast, B27-positive individuals affected with AS have a significantly lower *in vitro* responsiveness to *Klebsiella* (isolates 411, 427, 433, 462 and 468) as compared with B27-positive and B27-negative healthy controls. These observations, together with those of Ebringer *et al.*, suggest a possible cross-reactivity between *Klebsiella* antigens and HLA-B27, or the product of a closely linked gene. Alternatively, the lower lymphocyte responses to *Klebsiella* in AS patients may reflect an impaired processing of antigen by macrophages or the production of suppressor cells and/or suppressor factors by the lymphoid cells of affected individuals. These possibilities are now being studied.

In an attempt to gather further information on a possible cross-reactivity between *Klebsiella* and B27, we raised antibodies in rabbits to formalin-killed faecal isolates of *Klebsiella* (427, 433, 462 and 468) and to formalinised suspensions of *S. typhimurium* H specific and *S. typhi* H.

Rabbits (3–5 kg) were injected intravenously in the marginal ear vein with about 10^9 formalin-killed bacteria in normal saline (2 ml). Two weeks later, the animals received 10^9–10^{10} organisms in 2 ml of complete Freund's adjuvant (Difco) intramuscularly into multiple sites in the thigh and the nuchal skin. After 10–14 d the rabbits were boosted intravenously with 1 ml of bacterial suspension (10^8–10^9 organisms) in normal saline. Two weeks after the last injection, they were bled and the serum stored at −20 °C. In some cases the rabbits were boosted intravenously at 2- to 3-weekly intervals (for periods up to 6 months) with about 10^8 organisms in 1 ml saline, and bled 1 week after each injection.

Table 2 shows the results of a typical experiment on the cells of single individuals for each category. The data indicate that an antiserum to *Klebsiella* 427 lyses the lymphocytes of B27-positive patients with AS but not those of B27-negative individuals with active disease. By contrast, the lymphocytes of B27-positive and B27-negative unaffected individuals are not lysed by an antiserum to *Klebsiella* 427. Furthermore, the cytotoxic activity of antiserum 427 may be removed only by lymphoid cells from B27-positive patients with AS but not by lymphocytes from B27-positive or B27-negative normal controls. Antisera to other isolates of *Klebsiella* (468, 462 and 433) produced virtually no lysis of any of the target lymphocytes. Similarly, antisera to *S. typhimurium*, *S. typhi*, *Streptococcus* Group A and *Shig. dysenteriae* (both Wellcome) gave <20% ^{51}Cr release from the four categories of lymphocytes. Preliminary results (data not shown) indicate that antisera to *Y. enterocolitica* and *Staphylococcus* also fail to lyse the lymphocytes of affected and normal individuals. It is of interest that only one isolate of *Klebsiella* (427) gave a persistently high percentage ^{51}Cr release from the cells of B27-positive affected patients, whereas sera raised against the other three isolates produced only about 10% lysis. This suggests either that only some strains of *Klebsiella* cross-react with the putative B27-associated gene product in patients with AS or that *Klebsiella* 427 is more immunogenic in the rabbit than the other isolates tested. However, the latter possibility seems less likely, as rabbits boosted with isolates 468, 462 and 433 at 3-weekly intervals over a period of 4–6 months failed to produce detectable cytotoxic antibodies reactive with the lymphocytes of AS patients bearing the B27 allele.

Table 3 summarises the data on the reactivity of anti-*Klebsiella* 427 antiserum with lymphocytes from affected and normal individuals. The results strongly suggest cross-reactivity between some *Klebsiella* antigens and a gene product closely associated with HLA-B27 or possibly with genetically- or *Klebsiella*-modified B27 alloantigen complex in AS patients. The failure of anti-*Klebsiella* 427 antiserum to lyse cells from B27-negative AS patients suggests that B27 is an essential component of this hypothetical alloantigen–*Klebsiella* complex. Arnason *et al.*[10] have provided evidence that the predisposing determinant in AS may be either a modified B27 or a gene located on the Bf (properdin factor B) side of the HLA-B locus. This evidence was based on the rationale that if the predisposing locus were on the same side of the HLA-B locus as the Bf locus, B27-Bf haplotypes in those affected should be more uniform than in those not affected. On the other hand, if the B27 alloantigen predisposed directly there would be no reason to expect any difference in the incidence of the Bf alleles in the affected minority and the non-affected majority who carry the B27 allele. These authors found that patients with AS were Bf^s, whereas 23 of the controls (individuals with B27 but without symptoms) had Bf^f and 23 carried Bf^s.

In the murine system, the concept of 'altered self' has been extensively investigated by Zinkernagel *et al.*[11], using as their model the adoptive transfer of T-cell immunity to the bacterium *Listeria monocytogenes*. These workers showed that immunity to the bacterium can be successfully transferred only if the donor and recipient share a portion of the I (immune) region of the mouse H-2 complex. The T cells involved in this genetically restricted transfer seem to be specific either for a complex of 'self' in association with *Listeria* antigen or for bacterial antigen which is expressed on the cell surface and which is modified by I region-linked enzymes[12,13]. It remains to be determined whether a similar modification of the B27 allele, by an enteric organism, leads to the appearance of cytotoxic or suppressor T cells, and whether the activity of these cells is restricted by the human equivalent of the murine I region.

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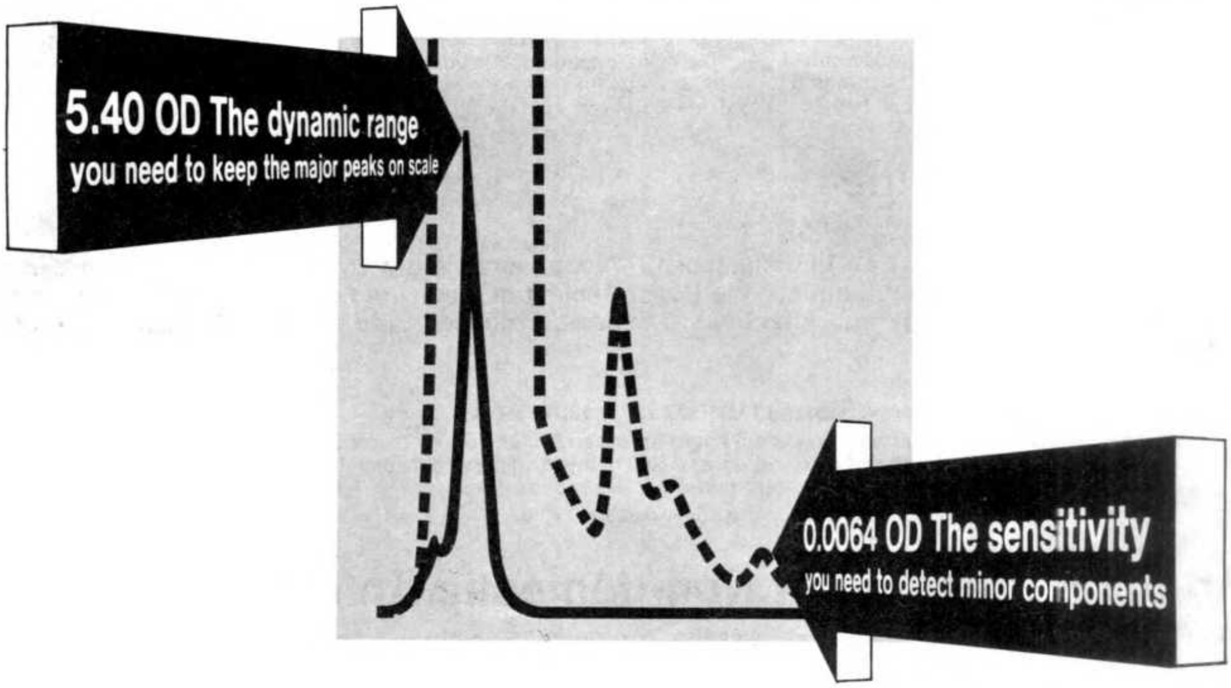
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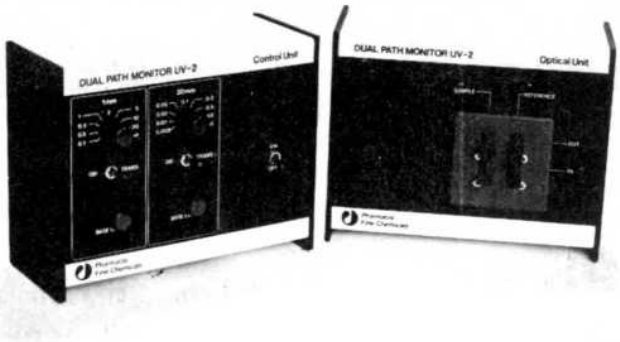

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This title was wrongly annotated in the December 7th 1978 issue

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Sussex school of perception

A. W. Still

Perceptions and Representations: The Theoretical Bases of Brain Research and Psychology. By K. Oatley. Pp. 262. (Methuen: London, 1978.) £7.

THIS is a non-technical exposition of what might be called (though probably not by its members) the Sussex school of perception. In 1959 Stuart Sutherland, following Lashley, set out the problems posed for any theory of perception, and many of his colleagues at Sussex, including the author of this book, contributed to the solutions subsequently offered. The problems arose because the invariants of perception are not reflected by invariants in the stimulus. Objects look the same from different aspects, for instance, and wherever their image falls on the retina, while the information in the stimulus may be drastically incomplete, but still recognisable, as in a cartoon figure. The Sussex solution is found in artificial intelligence, a branch of computer science which has created programmes to compute "picture descriptions". These programmes are based upon the spatial configurations of the parts of the picture. Physically different stimuli (or cues, the term preferred by Dr Oatley), such as pictures of the same scene from different aspects, can give rise to similar descriptions or representations, and can thereby receive a common classification (but they may not, depending upon the "purposes" incorporated in to the programme; thus there is an attractive freedom from control by the stimulus). Dr Oatley gives a very readable account of these views and his book will be of great value to students of psychology, as well as to anyone interested in the way computer science has influenced psychology.

He begins historically by tracing the background in reflex or stimulus–response theory. It is this theory which poses the problems recognised by Lashley, and which are unanswerable in its own terms. To develop more appropriate terms, Dr Oatley explores the idea of "emergent" properties, through an account of his own work on feedback mechanisms in hunger and thirst. Properties of a system "emerge" when they cannot be deduced from the "fundamental units" of the system. Thus "properties of the feedback loop, control, regulation and entrainable oscillation clearly emerge as properties of organisation of processes in a feedback loop, not the individual processes themselves". Likewise, representational properties of mind emerge from physical elements, and artificial intelligence is an analogue of this process. By anchoring the concept to feedback loops and computer technology, "emergence" loses its mystical tinge, and mind can emerge respectably as matter organised in particular ways.

There are some clear acounts of artificial intelligence programmes, which are made accessible to the general reader without being watered down. These programmes can provide answers to the problems posed by stimulus–response theory, but do they provide an accurate model of visual perception? The evidence is sparse. True, one need only observe one's own thought processes to see that many perceptual tasks (such as Shepard and Metzner's experiments on puzzles the solutions of which involve imaging rotations of pictured solids) are solved by mental manipulations that can be incorporated into artificial intelligence programmes. But is all visual perception of this kind? The answer given by this book is "yes", perception involves the solution of a kind of puzzle, as retinal cues are incomplete, degenerate, often ambiguous, and sometimes downright misleading. But are they?

A questionable premise to the argument is that the eye is like a camera. This view is encouraged by, and encourages, experiments on perception of pictures (rather than objects) which have to be interpreted or classified. If the eye is a camera (albeit far from perfect) the picture is imaged on the retina, so the interpretation of the picture is, in effect, an interpretation of the retinal information. But what is the image and what is the retinal information, for a monkey leaping through trees or a skier weaving his way amongst trees? If the static image of a picture projected on to the stationery eye of a laboratory subject is degenerate, what can we say about the information on the retina of a moving animal? If the computations in the interpretation of a static image are complex, what are the computations necessary for an image that is rapidly changing? Much more complex, obviously, and yet visually guided movement, often rapid and of great refinement, occurs throughout the vertebrates, and therefore amongst animals that show little ability to interpret pictures.

Perhaps artificial intelligence will find the answer to this; it is trying, and the difficulties are admitted, though not seriously considered by Dr Oatley. But there is a powerful alternative answer to the problem, also not seriously considered in this book. J. J. Gibson has shown how the invariants of perception might, after all, be reflected in the visual environment, especially if we take account of (rather than forget) the movements of the retina with respect to that environment. This involves giving up the eye-camera analogy and considering the environment in relation to an animal moving through it. The retinal projections will be transformed over time, and it is in these transformations that, if Gibson is right, important invariants of perception can be found. This is a truly new theory, whereas the picture description theory, far from being a modern reaction to stimulus–response theory as suggested by Dr Oatley, has its roots in the long philosophical history of perceptions being constructed out of sensations, sense-data, stimuli, or sensory inputs. The famous straight stick which looks bent in water is the direct ancestor of all the puzzle stimuli and ambiguous figures with which psychology textbooks abound. In both cases it is falsely concluded that because stimuli are sometimes misleading they must always be so, at least potentially.

These are possible criticisms of the Sussex school of perception, which, being a school, is naturally one-sided, and whether the criticisms are valid or not Dr Oatley's book remains an excellent statement of this one-sided theory. The shakiness of the theory's foundations are all the better exposed by the clarity of the book, especially as Dr Oatley himself expresses doubts in the last chapter. The doubts are general, about science as a paradigm for psychology. It has great value, but is limited, and does not, for instance, help in the conduct of personal relations. A suggested answer to these doubts lies not in less but more reliance on computers, in the form of the "convivial computing" of Papert's LOGO project. The aim of the project is to use computers as teachers, whose pupils will remain free of the hangups and fears of appearing foolish that human teachers frequently inspire. It is an impressive project, but surely not an answer to Dr Oatley's doubts. Yet what answer could he give? Perhaps there is none within his framework of physiological psychology and artificial intelligence. This may explain his choice of a quotation from Doris Lessing at the beginning of the book: "And when a book's pattern and the shape of its inner life is as plain to the reader as it is to the author—then perhaps it is time to throw the book aside, as having had its day, and start again on something new." ☐

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Protein turnover

Protein Turnover in Mammalian Tissues and in the Whole Body. By J. C. Waterlow, P. J. Garlick and D. J. Millward. Pp. 804. (North-Holland: Amsterdam, New York and Oxford, 1978.) $120; Dfl.276.

THIS is a scholarly book in the best sense of that term: Professor Waterlow and his colleagues know the subject matter thoroughly, think very clearly and write very well. It is an expensive volume, but the isotopically labelled materials you need to study protein metabolism in the whole animal are also expensive, and most workers in this difficult field could easily save the cost of the book by reading it carefully, and then designing their experiments more wisely.

A frontispiece to the book indicates a "scientific heritage" going back to the mid-eighteenth century, but the genealogy is not one of orderly succession. In the late 1930s Schoenheimer and his colleagues made an astonishing leap forward by showing that metabolic precursors of all kinds, and in particular amino acids, could be labelled with stable isotopes, and thus that their metabolism in whole animals and tissues could be followed. The technical problems which had to be overcome to carry out this work were so great that no other laboratory was able to carry on the torch, but as a result of Schoenheimer's work, biological scientists learned to regard the body less as a fixed machine which ran on dietary fuel, and more like a military regiment in which the structure is in dynamic equilibrium with the fuel—in this case, the recruits.

It has proved unexpectedly difficult to continue where Schoenheimer left off. In the past 20 years techniques for measuring both radioactive and stable isotopes have greatly improved. It is deceptively easy to carry out tracer studies with labelled amino acids, but all too often they are impossible to interpret. Good tracer studies need many skills, and if the investigator's grasp of biochemistry or mathematics is inadequate, mere technical accuracy is of no use. This book concentrates on the biochemistry and mathematics.

The first section (164 pages) is on processes: a review of protein synthesis, degradation, and amino acid pools and transport. The key sentence is: "No measurement of the rate of incorporation of a labelled amino acid into protein can be used to calculate the rate of protein synthesis unless the specific activity of the free amino acid *at the site of protein synthesis* is also known." The second section, on methods (263 pages) starts with a useful introduction to mathematical models. Non-mathematicians should read this, as the study of anything so complex as whole body protein turnover requires models, and the more you know about their advantages and limitations the more likely you are to use them appropriately. The next chapters deal with methods for measuring protein turnover in the whole body by observing free amino acid labelling, in individual tissues by observing incorporation of label into tissue protein, and some special cases such as isolated perfused organs.

The remaining 323 pages of text on results draws heavily on the work done by Professor Waterlow and his colleagues, and this concludes with a most stimulating chapter on directions of future research. There are great technical and intellectual challenges for the next generation of research workers, in a field ready for a new explosion of knowledge. The problem is that the body is much less homogeneous than a military regiment, or even an army, as there are many more types of protein, with different kinetics, than there are types of soldier. For those who take up the challenge to discover how and why protein is turned over in tissues and animals (including man) this book is an excellent guide. **J. S. Garrow**

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Seed development

Physiology and Biochemistry of Seeds in Relation to Germination. Vol. 1: Development, Germination, and Growth. By J. D. Bewley and M. Black. Pp. 306. (Springer: Heidelberg and Berlin, 1978.) DM90.

THE authors of this first volume of a two-volume work have concerned themselves with the physiology and biochemistry of seed development, germination and seedling growth. Approximately one-third of the book deals with seed structure and the development of the seed on the parent plant, and the remainder considers germination and seedling growth with particular attention to the mobilisation of reserves and the control of mobilisation. The interest in these topics and the use of seed and seedling material for much research in the plant sciences gives the subject of this volume a considerable importance for both teaching and research. Part of the work provides an excellent text for advanced students, one of its strongest points being its critical treatment of those dynamic aspects of seed development and germination, such as nucleic acid and protein synthesis and cereal aleurone layer studies, which have seen research work of a high quality in recent years. In areas in which there has been less distinguished recent work, such as the study of seed and seedling respiration and its control, the authors seem to have been rather less critical and somewhat over-generous in their assessment of the literature.

The authors' aim for comprehensiveness has resulted in the book having some rather dull sections, for example, on structure and on the chemistry and biochemistry of reserve materials, so that parts of the book have the character of a reference work, a feature of which is in keeping with the rather high price of the volume. Of course such a slim volume cannot really provide a comprehensive coverage of the subject and therefore each chapter is followed by some more general references so that the reader can fill out the topic. One vital and rather difficult topic has received very inadequate attention: the question of the measurement of germination, which receives less than two pages and exhortations to read the literature. Something more authoritative was called for here.

There are few errors in this attractively produced book. Most of the Tables and Figures have originated fairly directly from the literature and I found most value in those that had been specifically produced by the authors. In volume two I hope that the authors will give more scope to their illustrative talents and I look forward to the early appearance of this volume and to its treatment of the real problem, that of seed dormancy.

J. W. Bradbeer

J. W. Bradbeer is Professor of Botany at King's College, University of London, UK.

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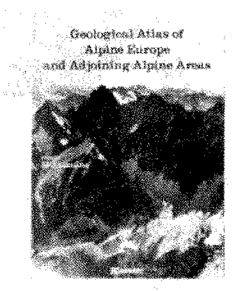

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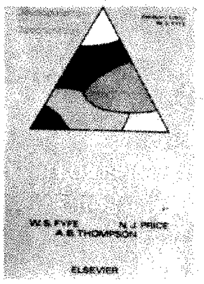

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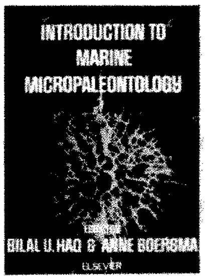

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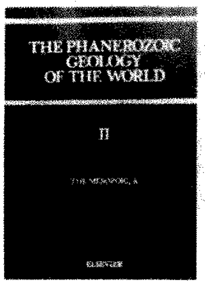

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Amino acid transport

A Review of Amino Acid Transport Processes in Animal Cells and Tissues. By J. Lerner. Pp. 234. (University of Maine at Orono Press: Orono, Maine, 1978.)

AMINO ACIDS are chemically stable and easy to assay. With rapidly expanding techniques of microperfusion, micropuncture and cell fractionation one might have been forgiven for thinking that most of what was worth knowing about these substances would soon be discovered. This has clearly turned out not to be the case for, despite a torrent of data produced in the past two decades, relatively little is known about the fundamental aspect of transport into and out of cells. The literature is massive, with up to 100 original articles and some half-dozen reviews appearing annually. Professor Lerner is to be congratulated therefore on tackling the mammoth task of reviewing this field and providing a valuable synopsis of current information and concepts.

He accomplishes his task in 177 pages of readable text which cites just over 900 references up to 1976. The index is excellent and the 15 chapters (some of which are a single page in length) are well laid out. Most data described have been derived from marine invertebrates, insects, parasites and mammals (with detailed considerations of transport in the gut, central nervous system and to a lesser extent the kidney), with the intact organism and isolated preparations considered in detail. There is adequate reference to other tissues and isolated cultured cells, with the differing specificity of particular transport systems according to organ and species suitably highlighted.

Perhaps more than anything else, the complex interactions of the amino acids themselves on their own transport and the paucity of synthetic non-competitive specific inhibitors, have hindered the elucidation of mechanistic details. However, of the effects known, those of hormones, mercury, nucleotides, amino acid analogues, vitamins and sugars are suitably described. The important influence of sodium ion concentration is tackled in detail, although rather surprisingly without reference to the elegant work by Ullrich and his collaborators on the isolated rat renal tubule.

Within the context of the whole organism there are interesting observations on the adaptation and regulation of transport and how transport changes during foetal development. Contributions from the clinical front include the inborn errors of metabolism, dietary deficiencies, malabsorption and starvation.

This volume is comprehensive but strictly a review. The theme is usually clear, but on occasions the book would benefit from an overall synthesis and some comment. Sometimes the argument becomes diffuse, points of interest or inconsistency are not highlighted, and there is little speculative comment. It is disappointing (but also indicative of our knowledge) that proposed mechanisms of transport only occupy 10 pages and there is no mention of any possible role for transmembrane peptides which are known to be important in other transport systems. However, despite these observations the work will undoubtedly be of considerable use to research workers in and students of biochemistry, neurochemistry, comparative physiology, nutrition, regulation biology and I think especially clinical science It should find a place in all laboratories working on amino acid metabolism.

C. W. J. Owens

C. W. J. Owens is Lecturer in Chemical Science at University College Hospital Medical School, London, UK.

Upper atmosphere, ionosphere and magnetosphere

Handbook of Astronomy, Astrophysics and Geophysics. Vol. 1: The Earth. 1. The Upper Atmosphere, Ionosphere and Magnetosphere. Edited by C. W. Gordon, V. Canuto and W. I. Axford. Pp. 412. (Gordon and Breach: London, 1978.) £35.

As this particular volume represents the first of forthcoming volumes on a very wide range of astronomy, astrophysics and geophysics, a few general comments may be in order on the general editorial matter. First, it is not clear whether or not this particular volume (volume 1) is supposed to cover the entire subjects of "The Upper Atmosphere, Ionosphere and Magnetosphere." If other volumes are planned for the above subjects, it should have been mentioned in an editorial note. How many volumes are planned? Is general subject matter for each volume decided? Or is this going to be a collection of review papers as they come along?

Secondly, the volume consists of six articles, a mixture of comprehensive articles covering a very wide range of topics and those covering rather specific topics. Thus, the general editorial policy for the general planning of this handbook is not very clear. Further, there does not seem to be a uniform format: some have an abstract, some do not. Some articles have one page for the title and some do not. For an extensive article, it would be helpful to have at least a Table of Contents on the cover page. Also, if it is going to be truly a *Handbook* it should have at least a subject index.

Among the articles in the first volume "Hydrogen in the Upper Atmosphere" by B. A. Tinsley, "Auroral Particle Precipitation . . . Observations" by B. A. Whalen and I. B. McDiarmid, and "The Physical Mechanisms of the Inner Van Allen Belt" by M. Walt and T. A. Farley are perhaps the most comprehensive and up-to-date ones in each subject area. The coverage of the article "The Equatorial Electrojet" by D. M. Cunnold is rather limited, and there is little mention of some of the recent developments, such as the relationship between the F region of the ionosphere and the equatorial electrojet. The article "Polar-Cap Absorption Obsevrations and Theory" by G. C. Reid does not cover observations of solar protons in interplanetary space and in the magnetosphere. The coverage of J. R. McAfee's article "Electron Plasma Resonances in the Topside Ionosphere" is reasonable for this limited subject.

Syun-Ichi Akasofu

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Acetylcholine synthesis

Acetylcholine Synthesis in Neurons. By S. Tuček. Pp. 259. (Chapman and Hall: London, 1978.) £15.

In recent years several excellent books on cholinergic mechanisms have been published (for example, *Biology of Cholinergic Function,* edited by A. M. Goldberg, I. Hanin, Raven: New York, 1976; *Neuromuscular Junction Handbook of Experimental Pharmacology,* **42,** edited by E. Zaimis, Springer: Berlin, 1976). As these books have covered many topics of cholinergic transmission in more or less detail, one might wonder whether there is a need for another book on acetylcholine. However, the present monograph aims to be an addition to, rather than a substitute for its forerunners, by giving a detailed analysis of many aspects of transmitter synthesis, a subject which has not yet received the degree of attention it probably deserves. In my opinion the book fulfils its aim in an elegant manner. In the first place it is extremely readable, if only it were for the fact that it measures less than 200 pages of text, in contrast to its more corpulent counterparts, where the busy reader may hesitate reading the full contents at leisure. If one takes into account its compact size the book is admirable in offering so much detail with such clarity. The author never seems to be in a hurry when explaining difficult matters; when there is no way out of a problem, as is often the case with conflicting evidence, he treats the subject with justified economy.

The book sets out with two chapters which nicely summarise what is known about choline acetyltransferase. Among other things the physical and enzymic properties of this protein are discussed, together with its presence in nerves and also in some non-nervous tissues, and its transport in cholinergic axons. Subsequently, there are two very informative chapters devoted to the supply of acetyl and choline moieties which finally form the acetylcholine molecule. The supply of choline and choline-containing substances to the nerves (with special attention to the brain) and the subsequent neuronal uptake of free choline are described, and the problem is discussed how, or rather in what chemical form, activated acetyl groups migrate through the mitochondrial membrane into the cell sap of the nerve terminal. These chapters are followed by a survey of what is known about the organisation and control of acetylcholine synthesis. This actually forms the body of the book, and is also the most interesting part.

In addition to synthesis, the storage and release of acetylcholine are briefly discussed, as there are good reasons to believe that storage and release are intimately connected with the synthetic process. According to the author the synthesis of acetylcholine is probably controlled by mass action, enough choline acetyltransferase being present to provide a rapid recovery from disturbances of equilibrium caused by release of transmitter. High affinity uptake of choline and release of activated acetyl groups by the mitochondrion may represent additional factors in the mechanism controlling acetylcholine synthesis. The turnover of acetylcholine in the living brain receives little attention in this chapter and perhaps some readers will regret this. A full discussion of this subject would have been redundant, because it has been extensively reviewed recently by I. Hanin and E. Costa in a chapter of *Biology of Cholinergic Function,* cited above. The last chapter of the book treats, among other things, the changes of choline acetyltransferase during development and ageing, and during Wallerian degeneration.

The book provides an extensive list of references (papers as recently as 1977 are referenced), which, so far as I have been able to judge, covers practically all that has been published in the field described in the book. It is the kind of reference list which makes the bulk of one's private file of references suitable for the dustbin.

Overall the book is a most valuable source of information on a topic which has not recently been reviewed elsewhere. Further it has the merit of being of educational value for investigators entering into the cholinergic field.

Peter Molenaar

Peter Molenaar is at the Department of Pharmacology, University of Leiden, The Netherlands.

Freshwater fish production

Ecology of Freshwater Fish Production. Edited by S. D. Gerking. Pp. 520. (Blackwell Scientific: Oxford, 1978.) £23.50.

In 1966 a symposium on *The Biological Basis of Freshwater Fish Production* was held at Reading University, England, and the proceedings were published by Blackwells in 1967 as a volume carrying the same title. The book was in great demand and has sadly been out of print for some time. However, the overwhelming response to that volume stimulated the production of the present book. The title has been changed and, although it is still recognisable as a 'reprint' of the earlier edition with many of the same authors simply revising and up-dating their previous articles, it does have some new chapters, some new authors and a somewhat different approach. In addition some of the authors appearing in the first edition are absent from this one. Dr Shelby Gerking has been the editor of both volumes and has done a remarkably fine job. The present *Ecology of Freshwater Fish* is a much better work than its predecessor and, as the former was an excellent text, one can appreciate the high opinion the reviewer has of this present edition.

The book is divided into four main areas: vital statistics of fish populations; the fish population and its food supply; competition and social behaviour influencing production; and the contribution of fish production to human nutrition and well-being. The last section is most welcome, and one worthy of the lengthy treatment it receives this time. It includes contributions on fish yield assessment of large lakes and reservoirs, the growing science of aquaculture, ecological aspects of warmwater fishpond management, and the contribution of freshwater fish to human food, the latter containing a most valuable collection of statistics and up-to-date information.

In the scheme of fish production, information on mortality is required, and one serious omission in this otherwise very complete book is any consideration of mortality from disease, parasitism and predation. Certainly the role of predaceous fish in ecosystems is dealt with in some length, but one would have thought that two papers, one on disease and parasitism, and, one on predation by other animals, would have been a 'must'. There is considerable published work on the effects of disease and predation on both production and yield. Even in the first edition a paper on predation on fish by other animals was included, but for some reason this has been omitted this time.

The book is up to Blackwells usual high quality of production, and only one figure (Fig 127, p320) is sub-standard. It should be in the library of every university department of biological sciences and on the shelves of freshwater fishery staff. It would also be a useful investment for undergraduate as well as postgraduate fisheries students.

Derek Mills

Derek Mills is Senior Lecturer in freshwater ecology and fisheries management at the University of Edinburgh, UK.

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POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for the po of Postdoctoral Research Associate t work on a project funded by th Medical Research Council. The proje is concerned with the nature of th bacterial cell surface and will invol a study of the effects of varied wa composition on the ability of bacter to interact with a variety of surfac probes including bacteriophages, ant bodies and lectins.

The salary is in range 1A (£3,8 to £4,382 per annum) plus supe annuation benefits. Applications, i cluding a curriculum vitae and th names and addresses of two refere should be sent to Dr A. R. Archibal Microbiological Chemistry Researc Laboratory, University of Newcast upon Tyne, Newcastle upon Ty NE1 7RU, from whom further info mation may be obtained. 8983(A)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF MATHEMATICS

Applications are invited for

TWO LECTURESHIPS

in Mathematics. Candidates may ha special interests in any branch of pu or applied mathematics. Salary sca £3,909 to £7,754 plus £450 Lond Allowance. Applications, includi the names of two referees, should addressed to the Assistant Secreta (Personnel) (N), University Colle London, Gower Street, London WC 6BT, from whom further particula may be obtained. 8954(A)

ST MARY'S HOSPITAL

Praed Street, W.2

MEDICAL LABORATORY SCIENTIFIC OFFICER (QUALIFIED)

is required in the Department Chemical Pathology to assist with research project, for three years, i volving the investigation of stero hormone receptors in human tiss and radioimmunoassay.

Salary scale £3,615 per annum risi to £5,034 per annum fully inclusive.

Applications in writing givi details of previous experience plus t names and addresses of two refere to the District Personnel Officer, Mary's Hospital, Praed Street, Lond W.2. 8972(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

RESEARCH TECHNICIAN GRADE 4

Experienced research technici required for last 18 months of M.R.C. projejct to study the regu tion of initiation of protein synthe in cultured cells and cell-free syste B.Sc. or N.H.C. or equivale familiarity with techniques molecular biology and/or tissue cult an advantage. Emoluments £3,230 £3,317 per annum (under revie Application forms from the Establi ment Officer, St George's Hospi Medical School, Cranmer Terra London SW17 0RE. Closing d **January 25, 1979.** 8947(A

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

Nairobi, Kenya

Applications are invited from suitably qualified persons for the following positions.

1. RESEARCH SCIENTIST (TERMITE RESEARCH PROGRAMME) REF: SCI/78/27

Qualifications

Ph.D. with 5 or more years of postdoctoral experience in biological or physiological sciences and a demonstrable experience in research on the physiology of insect development. Training and experience in insect sensory physiology and behaviour, and evidence for excellence since doctorate training, will be a distinct advantage.

Responsibilities:

The successful candidate will carry out research on the physiology of caste differentiation among higher termites, with special reference to possible involvement of the endocrine and exocrine systems.

POSTDOCTORAL RESEARCH FELLOW IN PLANT PHYSIOLOGY OR BIOCHEMISTRY (BASES OF PLANT RESISTANCE TO INSECT ATTACK). REF: SCI/78/28.

Qualifications:

Young postdoctoral scientists, newly graduated, whose experience is in plant physiology, plant biochemistry or relevant sciences. High-quality research for Ph.D. and evidence for excellence since doctorate work, will be a distinct advantage.

Preference will be given to gifted young scientists from developing countries.

Responsibilities:

The successful candidate will carry out research in the field of plant physiology and biochemistry vital to plant/insect relationships. Willingness to collaborate with colleagues in other disciplines, especially in insect sciences, will be of paramount importance.

GENERAL INFORMATIONS FOR ALL POSITIONS:

Salary:

Negotiable and include attractive benefits.

Applications giving detailed information on general education, professional qualifications, experience, marital status, age, present terms of employement (including salary) names and addresses of four referees (including one personal), and photostat copies of relevant certificates and diplomas should be addressed to:

Administrative Manager
I.C.I.P.E.
P.O. BOX 30772,
NAIROBI, KENYA.

To reach him not later than 15th January 1979.

8995(A)

NATURE CONSERVANCY COUNCIL

Higher Scientific, Senior Scientific and Principal Scientific Officers

SALARY SCALES

Higher Scientific Officer	£4,101 — £5,448
Senior Scientific Officer	£5,154 — £6,898
Principal Scientific Officer	£6,609 — £8,461

Applications are invited for the following posts in the NCC Field Unit based in Scotland (Edinburgh) and the Chief Scientists Team based in England (Huntingdon).

BACKGROUND

(a) The functions of the FIELD UNIT is to develop a co-ordinated programme of survey and monitoring in SCOTLAND in conjunction with Country HQ, Regions and the Chief Scientist by carrying out appropriate survey and monitoring projects and to synthesise and interpret the results so as to facilitate maximum use of the information by all staff within the organisation.

(b) THE CHIEF SCIENTISTS TEAM provides specialist scientific advice on all aspects of NCCs work and is responsible for the research programme. Members of the team are responsible for ensuring that appropriate research is carried out either by means of contracts placed with research organisations or by the team itself and other NCC staff.

POSTS

1. Field Unit Leader

SCOTLAND (SSO)

A biologist is required, with considerable experience of botanical ecological survey work.

2. Coastal Ecologist

SSO/PSO—CHIEF SCIENTIST TEAM

Substantial knowledge of the flora and vegetation of coastal habitats and experience of associated conservation problems essential.

3. Vertebrate Zoologist

(HSO)—CHIEF SCIENTIST TEAM

Special experience with mammals needed.

AGE AND QUALIFICATIONS

All candidates should have a first or second class honours' degree, or equivalent, in biology, botany, zoology, geology or geography.

Higher Scientific Officer: Candidates should be under 30 years of age, and have at least two years relevant post-graduate experience.

Senior Scientific Officer: Candidates must be at least 25 and under 32 years of age and have at least 4 years relevant post-graduate experience.

Principal Scientific Officer: Candidates should normally be between 30 and 50 years of age.

All posts will ential extensive travelling and possession of a current driving licence is essential.

Application forms and further details are available from Recruitment Section, Nature Conservancy Council, Godwin House, George Street, Huntingdon, Cambs PE18 6BG (Tel: 0480 56191). Closing date for completed application forms is February 2, 1979. Interviews will be held in March 1979.

8952(A)

UNIVERSITY OF SINGAPORE

CHEMICAL ENGINEERING

PROFESSORSHIP

Applications are invited for appointment as Professor in the Department of Chemical Engineering. The successful candidate should have considerable teaching, industrial and administrative experience. Gross monthly emoluments in the range from S$3,745 to S$5,775 approx., the initial amount depending on the candidate's qualifications and experience. In addition, the University pays a 13th month annual allowance of one month's salary in December of each year. Leave, subsidised housing, medical and provident fund benefits are available. Candidates should write to: **The Registrar, University of Singapore, Kent Ridge, Singapore 5,** giving curriculum vitae (bio-data), with full personal particulars and also the names and addresses of three referees.

9023(A)

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university of wales

university college of swansea

SENIOR RESEARCH ASSISTANT

HIGH PERFORMANCE SEMI-CONDUCTOR OXIDES

Senior Research Assistant is required for an S.R.C. grant investigating chemical changes in semi-conducting oxides exposed to reducing environments, with the aim of developing gas detectors which will exhibit specificity to certain gases. Instruments which incorporate these detectors will be designed within the **Electrical Engineering Department.**

Applicants should have a good honours degree and preferably research experience in solid state chemistry or an immediately relevant subject.

The appointment is for three years commencing as soon as possible and the remuneration will be up to £4,382 per annum plus U.S.S./U.S.D.P.S. benefits.

Prospective candidates may contact Dr K. J. Gallagher of the Chemistry Department for further particulars and application forms returned as soon as possible.

SENIOR RESEARCH ASSISTANT/RESEARCH ASSISTANT

Applications are invited from engineers/scientists for the post of **Senior Research Assistant/Research Asistant in the Department of Chemical Engineering** to work on a research programme supported by the Natural Environment Research Council. The successful applicant will undertake work associated with the rheological properties of cohesive suspensions occurring naturally in the marine environment.

The appointment, which will be from the soonest date that can be arranged to March 31, 1980, will be on a scale up to £5,367 per annum, together with U.S.S./U.S.D.P.S. benefits.

Closing date for applications, Wednesday, January 31, 1978.

Further particulars and application forms for the above posts (two copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP to whom they should be returned by the above date.

8994(A)

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANT

Applications are invited for the posotion of RESEARCH ASSISTANT to work with Dr J. Kay on the regulation of a neutral, muscle protease by endogeneous inhibitors. Techniques will involve enzyme and inhibitor purification, affinity chromatography, antibody production, radiolabelling, etc.

The post is available for three years financed by a grant from the Science Research Council. The salary will be in the range: £3,384 to £3,883 (under review). Duties to commence as soon as possible.

Candidates should have a good Honours Degree in biochemistry or a related subject and research experience in protein chemistry/enzymology/radioimmunoassay procedures would be an advantage.

Applications (2 copies), including a curriculum vitae, together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL. Closing date, **January 31, 1979. Please quote reference 1668.**

8961(A)

UNIVERSITY OF LONDON

INSTITUTE OF PSYCHIATRY

RESEARCH ASSISTANT

Applications are invited from recen graduates in pharmacology, bic chemistry or a related discipline, t undertake a behavioural and bic chemical investigation in rodents c the potential use of new drugs for th treatment of Parkinson's disease.

The position, which is supported b the Medical Research Council, i tenable for one year in the firs instance, and may be renewed for u to three years.

Salary £3,214 to £3,684 per annur plus £450 London weighting.

Applicants may visit the Departmen (Department of Neurology) (Professo Marsden and Dr Jenner) by prio arrangement. Phone: 01-703 5411 E 245.

Application forms available from th Secretary, Institute of Psychiatry, D Crespigny Park, Denmark Hill, Londo SE5 8AF. Phone: 01-703 5411 Ex 228, quoting ref: CM/N.

8990(A)

LINCOLN COLLEGE

(University College of Agriculture)

New Zealand

CHAIR OF SOIL SCIENCE

The Council of Lincoln Colleg invites applications for appointment t the above mentioned Chair, resultin from the impending retirement of Pr fessor T. W. Walker. It is th Council's desire to have the appointe take up duties towards the end of 19 or early in 1980.

The Chair in Soil Science is one 15 at the College and the Council anxious to fill the position with a appointee of high standing because the significance of this Chair, not on to the College but also to New Zealan agriculture.

Applicants should hold an advance degree in Science, Agricultural Scien or Soil Science, have had research e perience in some branch of soil scien or soil resources, such as soil fertilit soil chemistry, soil physics, soil biolo and pedology, and have had some e perience in teaching, communicatic or extension.

The successful applicant will appointed as Head of Department ar will be responsible to the Colle Principal for the administration of t Department.

The appointment will be mac within the existing range of professori salaries NZ$23,012 to $29,188 p annum, the commencing salary bei determined by the College Counci Academic salaries are at present und review.

Expenses of appointment reimburs up to specified limits. New Zealar Government Superannuation is ava able.

Further details and general Con tions of Appointment are obtainab from the Secretary General, Associ tion of Commonwealth Universiti (Appts.), 36 Gordon Square, Lond WC1H 0PF, or from the Registrar the College.

Applications close on **February 1 1979.**

8950(A)

UNIVERSITY OF NOTTINGHAM

School of Agriculture

DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES

Applications are invited for the p of Senior Demonstrator to assist l tures and demonstrators in Anin Physiology. Applicants should trained in vertibrate physiology veterinary science and should pref ably have gained a Ph.D. Resear facilities in animal physiology available. The post is for two yea salary on the scale £3,384 to £3, per annum.

Full curriculum vitae includ names and addresses of two refer should be sent to the Staff Appoi ments Officer, University of Notti ham, University Park, Nottingh NG7 2RD, quoting Ref. No. 649, later than January 21, 1979. Furt **details may also be obtained from above address.**

9003(A

Petroleum Engineering

For Major Development of Operations in Iran
c $33,000 to c $37,000 pa nett

OSCO – The Oil Service Company of Iran, (a consortium of 14 international oil companies) is engaged in a major programme of oil and gas production development in South West Iran on behalf of the National Iranian Oil Company. In order to accomplish this task we seek to strengthen our team of senior professionals by making the appointments detailed below.

Initial contracts are for two years. Salaries, according to seniority and experience are paid in US dollars nett of Iranian income tax. Benefits include married or single status accommodation, free medical facilities, life insurance, educational assistance and a bonus on termination of contract.

Project Leader-Reservoir Systems c $37,000 p.a.

Responsible to the Head of Reservoir Systems, you will be leading a small team maintaining and developing reservoir simulation models in order to assist the engineers to determine optimum exploitation strategies. You will control computer time worth approximately $100,000 p.a. using IBM and CDC hardware and programmed in FORTRAN. *(Ref F 581/N)*

Senior Reservoir Engineer c $36,000 p.a.

This is an important specialist appointment requiring advanced knowledge and originality in the application of reservoir analysis techniques, since the Iranian reservoirs present a variety of unusual technical problems. Working largely on your own initiative, you will be responsible for conducting reservoir studies to establish and predict performance and behaviour under various producing conditions. *(Ref F 440/N)*

Senior Petrophysicist-Methods c $33,000 p.a.

You will be responsible for determining from logs and format samples, the physical properties and fluid content of oil reservoirs. You will also play an active part in the training and guidance of well site and office personnel in the petrophysical implications related to their own functions. *(Ref F 259/N)*

Senior Petroleum Engineers - Processing c $33,000 p.a.

Who will be involved in the planning and review of present and future crude oil processing operations within OSCO. This will include forecasting the qualities of stabilized crude and separation gasses under various conditions and the effects of blending crudes from various field or reservoirs. *(Ref F 009/N)*

Candidates for these specialist appointments will need to be graduates in appropriate disciplines – Petroleum Engineering, Chemical Engineering, Physics or Mathematics – aged 30 or over and have had a minimum of eight years' professional experience in the oil and gas industry.

Please write giving full details of your age, education, career history and salary progression to date, quoting appropriate reference number to:
F. R. Wilcockson, Director,
Whites Recruitment Limited,
72 Fleet Street, London EC4Y 1JS.

IRANIAN OIL SERVICES LTD.

8964(A)

Assistant Plant Pathologist

The Agricultural Science Service of ADAS provides farmers and growers with a wide range of vital technical information and advice which is supported by investigation and development work carried out from 12 regional centres across the country. The assistant will join the Plant Pathology Department in Reading, where the work includes advisory, investigational, statutory and promotional activities.

The assistant plant pathologist appointed will primarily be concerned with plant diseases caused by fungi, viruses and bacteria, and also with plant diseases which cannot be attributed to known causes. The successful candidate will supervise the field investigational programme of the department. This will involve frequent field visits to layout experiments, assessing disease incidence using disease assessment keys and writing preliminary reports on the investigation. In addition, the assistant plant pathologist may be expected to supervise the laboratory examination of a wide range of samples and plant materials derived from both advisory and investigational activities and to liaise with farmers in sample collection, promotional activities and surveys.

Candidates, normally aged at least 25 should have GCE passes or Grades A, B or C in English Language and four other subjects, two of which must be in an appropriate scientific subject at 'A' level, *or* HND/HNC in plant pathology or applied biology *or* an equivalent qualification. At least 5 years relevant experience, preferably including practical experience of the plant pathological examination of plant material, and the ability to organise and supervise staff is essential. Candidates must hold a full valid UK driving licence.

Salary, starting at £4,125 rises to £5,205. There are promotion prospects and a non-contributory pension scheme

For further details and an application form (to be returned by 26 January, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). **Please quote ref: S/5006.**

8945(A)

MAFF:
AGRICULTURAL DEVELOPMENT
ADVISORY SERVICE

UNIVERSITY OF DURHAM

DEPARTMENT OF CHEMISTRY

Applications are invited for chemists or chemical physicists for an S.R.C Postdoctoral Senior Research Assistantship for 17 months from as soon as possible to work in collaboration with Dr J. Yarwood on the interaction and dynamics of hydrogen bonded systems in the liquid phase. These studies which include infrared, far-infrared, Raman and neutron scattering studies are also supported by a N.A.T.O research grant and would be carried out at Durham, the Technical University, Braunschweig and at A.E.R.E Harwell. Some experience in one branch of molecular spectroscopy is essential and an interest in the chemical physics of molecular motions and interactions in the liquid phase would be an advantage.

Initial salary £3,883 or £4,133 per annum on National Research Range 1A plus superannuation.

Applications (three copies) naming three academic referees should be sent by January 22, 1979, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE

8904(A)

CLARENDON LABORATORY

Oxford

Synchrotron Radiation

Applications are invited for a Postdoctoral appointment to investigate the application of Synchrotron Radiation to X-ray crystallography. The work will involve the design and implementation of two energy-dispersive diffractometers, together with associated computer software, for the new storage ring source under construction at the Daresbury Laboratory. In addition, the appointee will be expected to join in research into other types of X-ray crystallographic experiments at Daresbury and possibly at synchrotron sources elsewhere Applicants should have experience in X-ray diffraction, instrumentation and computing.

The salary scale (under review) will be £3,383 to £6,555, superannuable under U S.S. and the appointment will be for up to 2½ years, depending on age and date of appointment.

Curriculum vitae should be sent to Dr A M. Glazer, Clarendon Laboratory, Parks Road, Oxford OX1 3PU, U.K., to whom references from two referees should be sent directly.

9013(A)

ALFRED HOSPITAL

Melbourne, Australia

ENDOCRINE SCIENTIST

The Ewen Downie Metabolic Unit at The Alfred Hospital, Melbourne, which is affiliated with Monash University, requires a Ph D. Scientist with expertise in Radioimmunoassay, as well as experience with receptor techniques, hormone purification or affinity chromatography. The unit has both clinical laboratory responsibilities with service and research functions. The successful applicant will be able to conduct an active research programme related to either thyroid hormones, the renin-angiotensin system or adrenal steroids

The position involves responsibility for a radioimmunoassay laboratory in addition to the research programme.

Salary dependent on qualifications and experience

For further information send curriculum vitae including bibliography and summary of research interests together with names of three referees to. The Director, Ewen Downie Metabolic Unit, Alfred Hospital.

Official applications should be forwarded to the Director of Personnel Services, Alfred Hospital, Commercial Road, Prahran, 3181, Victoria, Australia.

9018(A)

UNIVERSITY OF OXFORD

E. P. ABRAHAM PROFESORSHIP OF CLINICAL PATHOLOGY

The electors intend to proceed to an election to the E P Abraham Professorship of Chemical Pathology with effect from October 1, 1980. The stipend of the professor is at present £10,828 per annum.

Applications (nine copies), naming three referees but without testimonials, should be received not later than March 26, 1979 by the Registrar, University Offices, Wellington Square, Oxford OX1 2JD, from whom further particulars may be obtained.

8959(A)

INSTITUTE OF Cancer Research (Sutton). A postdoctoral Scientist is required to fill a post that falls vacant on January 1, 1979 in the Radiotherapy Research Department of the Institute of Cancer Research The work of the Department involves the study of the biological effects of radiation and cytotoxic drugs on mouse tumours and on human tumour xenografts, making use of tissue culture techniques to assess in vivo responses Familiarity with mammalian radiobiology, tissue culture, or pharmacology would be an advantage The appointment will be for an initial period of 3 years, with a possibility of extension. Salary in the range £3,883 to £5,129 per annum plus London Allowance of £450 per annum. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref 300/G/136

8966(A)

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT FOR THEORETICAL ANALYSIS OF DNA STRUCTURES

Applications are invited from theoretical chemists or those with related interests to collaborate with Dr B Robson in the theoretical analysis of DNA and smaller analogues using potential function refinement, *ab initio* quantum mechanics and solvent Monte Carlo techniques. A sound knowledge of FORTRAN is required for the adaptation of established computer programmes The appointment is from one to three years at an initial salary of up to £4,382 per annum Superannuation Applications, including curriculum vitae and naming three referees, should be sent to Dr B Robson, Department of Biochemistry, The University, Manchester M13 9PL

9004(A)

UNIVERSITY OF SUSSEX

SCHOOL OF MATHEMATICAL AND PHYSICAL SCIENCES

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a Postdoctoral Research Fellow in the field of Low Temperature Solid State Physics The successful applicant will join a small research team, which is sponsored by the S.R.C and is concerned with experimental studies of many-body effects in metals using quantum oscillations.

The appointment will be for three years, salary on the scale £3,883 to £6,555 per annum (under review), with superannuation benefits

Applications to The Chairman of Physics, School of Mathematical and Physical Sciences, University of Sussex, Falmer, Brighton BN1 9QH, from whom further particulars can be obtained.

9005(A)

Avon Area Health Authority (T)
Southmead Health District

SOUTHMEAD HOSPITAL BRISTOL

Applications are invited for a post of

BASIC GRADE BIOCHEMIST

in the Department of Clinical Chemistry. Preference will be given to candidates having appropriate experience and a postgraduate qualification Opportunities for wide experience are available, but there will be particular emphasis on training in paediatric aspects

Application forms from District Personnel Officer, Southmead Hospital, Bristol BS10 5NE (Tel Bristol (0272) 505050, Ext 162) and should be returned by January 31, 1979

For further information D J. B. Holton (0272) 505050 Ext. 52, or Dr D J Goldie (Ext 51) should be contacted.

8982(A)

FACULTY POSITIONS— BIOCHEMISTRY

Several tenure-track positions a
level to be determined by qualificati
are open in the Biochemistry Dep
ment, School of Medicine, at the U
versity of North Carolina at Cha
Hill We seek to establish expertise
any of the following research ar
Culture, genetics, and differentia
of eukaryote cells; cell surface in
actions; mammalian gene trans
However, we will consider outstan
candidates regardless of resea
interests

Excellence in teaching is expec
Send curriculum vitae, the names
three references, and an outline of
search plan, by March 15, 1979
Dr Mary Ellen Jones, Chairwom
Biochemistry Department, Unive
of North Carolina, Chapel Hill, N
27514 **We are keenly interested**
attracting outstanding women an
minority scientists to this departm
Equal Opportunity/Affirmative Ac
Employer 8970(

UNIVERSITY OF TORON
SCARBOROUGH COLLE

Canada

Computer Science Group in
applications for tenure st
ASSISTANT PROFESSOR app
ment, available July 1, 1979 P
Computer Science essential with
ference given to areas of data b
computer graphics, program
languages, or operating syst
Duties include undergraduate
graduate teaching and research
curriculum vitae and names of
referees to Chairman, Ph
Sciences Division, Scarborough
lege, West Hill, Ontario, Ca
M1C1A4. 9010

PHYSICISTS

Department of Applied Science
King Abdulaziz University
Jeddah, Saudi Arabia

Physicists of all ranks are required to work in the Department of Applied Science at the King Abdulaziz University in Jeddah, Saudi Arabia.

We are offering attractive salaries and furnished housing accommodation as well as two months annual leave for the successful candidates and their families (maximum 4) with round trip air travel paid for.

Please send your application by air mail enclosing three letters of reference to:

Dr. Mohammed M. O. Jamjoom,
Dean of Engineering—Kaau,
P.O. Box 1540, Jeddah, Saudi Arabia

9014(A)

ORGANIC CHEMIST–MASS SPECTROSCOPIST

A postdoctoral research fellow

is needed for one year to be in charge of our new mass spectrometer/data (MS 50/Stereo DS50SM/DS50S) system and to supervise our technical assistant.

The work offers interesting structural, analytical and biochemical problems mainly in the natural product field and is conducted in collaboration with several organic research chemists. It involves predominantly application of capillary column GC—high resolution MS in the EI and CI modes as well as ion structural studies.

Applications are invited from candidates with relevant experience in the fields of organic chemistry and mass spectrometry.

The annual salary is about £9,000 and assistance will be given with relocation expenses and in obtaining accommodation.

Please write to Professor C. R. Enzell, Chemical Research Department, Swedish Tobacco Co., Box 17007, S-104 62 Stockholm, Sweden, enclosing a brief *curriculum vitae and references*, before January 31, 1979.

8974(A)

Portsmouth Polytechnic
DEPARTMENT OF
BIOLOGICAL SCIENCES

RESEARCH ASSISTANT

for two year contract initially, to work on growth in marine molluscs Applicants should have a 1st or 2nd class honours degree in a biological subject and be interested in the broader applications of their research work to mariculture. Candidates would be expected to register for an M.Phil (C.N A.A.)

Salary scale. £2,589 to £2,643 per annum.

Application forms and further details from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ, to whom completed applications should be returned by January 18, 1979.

8973(A)

UNIVERSITY OF
LIVERPOOL
DEPARTMENT OF
BOTANY

Mineral Industries Research
Organisation Research Project

SENIOR RESEARCH ASSISTANT

Applications are invited for one post of Senior Research Assistant to develop guidelines for the mineral extraction industry for the establishment of vegetation on metalliferous wastes by laboratory experimentation and literature survey Applicants should have research experience in plant ecology, soil science, or geochemistry, and be interested in working in collaboration with industry The post is tenable for a minimum of 18 months at a salary of £3,883 or £4,133. Applications should be received by the Registrar, The University, P O Box 147, Liverpool L69 3BX, not later than February 23, 1979, from whom further particulars may be obtained Quote Ref· RV/435/N. 9000(A)

COMMONWEALTH
AGRICULTURAL
BUREAUX
Vacancy for

SCIENTIFIC INFORMATION OFFICER

at the
Commonwealth Bureau of
Horticulture and
Plantation Crops
East Malling Research Station
Nr Maidstone, Kent

Duties Preparing abstracts in English for the journal *Horticultural Abstracts*, which covers world scientific literature in this field, indexing and answering scientific enquiries.

Qualifications A degree in horticulture, agriculture, botany, or related subject, plus a reading knowledge of at least one European language (German preferred) and the ability to write good English.

Salary: In the scale £2,839 to £5,448, plus a compensatory allowance (taxable but not superannuable) of 3 3 per cent of basic salary to offset personal contribution to superannuation scheme Starting salary according to qualifications and experience Promotion to higher scales on merit

Application forms and full particulars from the Executive Director, Commonwealth Agriculture Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN

Closing date for applications January 29, 1979. 9001(A)

BEHAVIORAL PHYSIOLOGY

The Department of Biology, University of Chicago, invites applications for a faculty position in Behavioral Physiology. For a description of the position and application instructions, write Behavioral Physiologist Search Committee, Department of Biology, University of Chicago, Chicago, Ill 60637 An Equal Opportunity/Affirmative Action Employer. 9024(A)

Biochemist (class 3)

Australian Department of Veterans' Affairs Victorian Branch

Applications are invited from Biochemistry Graduates for the above position, which is the senior non-medical appointment in the Biochemistry Department, Repatriation General Hospital, Heidelberg, Victoria The Hospital, of 550 beds, is a teaching Hospital of the University of Melbourne, and is situated in a pleasant 21 hectares setting in the northern residential suburbs of Melbourne; the Department is modern, spacious and well equipped The successful candidate will be responsible to the Senior Specialist, Biochemistry for all aspects of the Hospital's routine clinical chemistry Support staff comprises 14 Graduate and Technical personnel including two Class 2 Biochemists (Senior Biochemist equivalent), plus Trainees.

Further information may be obtained from Dr J. Green, Senior Specialist, Biochemistry, Repatriation General Hospital, Heidelberg, Victoria, 3081, Australia.

Salary

$A17,116 to $A18,910.

At present rate of exchange, £1 = $A1.71

Qualifications

An appropriate degree or diploma in Science or equivalent qualification Wide experience in clinical chemistry, and a higher qualification in either clinical chemistry or biochemistry desirable

Conditions

Four weeks annual recreation leave Cumulative sick leave Participation in the Australian Government Retirement Benefits Scheme Three months long service leave after 10 years service Fares to Melbourne for the appointee and family and an allowance until permanently housed may be paid

Application

Written applications, giving full details of qualifications and experience and listing 2 or 3 referees should be forwarded to

The Medical Superintendent,
Repatriation General Hospital,
Banksia Street,
HEIDELBERG VIC 3081
AUSTRALIA

by 27th January, 1979 8968(A)

Australia

Research Fellow in Microbiology

The Pharmaceuticals Division of ICI Ltd wishes to appoint a Research Fellow for a period of two years to study the adhesion of micro-organisms to epithelial surfaces. This work will form part of the Division's overall programme of research in infectious disease embracing viral, bacterial and fungal infections.

Applications are invited from people with research experience in microbial biochemistry, general microbiology or cell biology with a particular interest in the interaction between prokaryotes and eukaryotes.

Our modern research laboratories are situated in pleasant surroundings but within easy reach of main road and rail routes. Conditions of service and salary are excellent for this particular position.

Please write giving details of qualifications and experience to:

ICI

Mr M F Losse Personnel Officer
ICI Ltd
Pharmaceuticals Division
Mereside Research Laboratories
Alderley Park
Macclesfield Cheshire 9008(A)

UNIVERSITY OF OXFORD
DEPARTMENT OF FORESTRY
COMMONWEALTH FORESTRY INSTITUTE
TECHNICIAN Grade IV
£2,955 to £3,402 (under review)

A vacancy exists for a technician to assist with a research project concerned with the wood properties of tropical pines, for which funds are available until March 31, 1980.

A minimum of two 'A' levels (or equivalent) in appropriate subjects is required Duties will include cutting and preparing wood samples, X-ray photography and the use of computers

Applications giving age, qualifications and experience and naming two referees should be sent before January 14, 1979 to — The Administrator, Commonwealth Forestry Institute, South Parks Road, Oxford OX1 3RB 9022(A)

UNIVERSITYOF GLASGOW
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH

Applications are invited for a Postdoctoral Research Assistant to investigate substitution and ^{18}F exchange reactions of uranium (VI) and uranium (V) fluorides in collaboration with Professor D W. A Sharp and Dr J M. Winfield. The post, supported by the S R C, is tenable for three years, and is available from March 1979 Starting salary (1A Scale) in the range £3,883 to £4,382 per annum plus U S.S

Applications, with the names of two referees, should be sent as soon as possible to Dr J M Winfield, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, from whom further particulars may be obtained.

In reply please quote Ref. No 4344M. 9002(A)

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY
RESEARCH ASSISTANT
(Grade 1A— Salary £3,383 to £6,555)

Applications are invited for a Research Assistant, supported by the Cancer Research Campaign, to assist with research into the biochemical mechanisms of virus growth. Experience with mammalian tissue culture would be an advantage. Applicants should possess a degree or equivalent qualification.

Applications, in writing, together with the names and addresses of two referees, to the Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. 8960(A)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF CANCER STUDIES

A postdoctoral Research Fellow is required to join a team of scientists studying the proteins of normal and transformed cells The project will be of three years duration and the successful candidate will be expected to have a good biochemistry background and a working knowledge of protein separation techniques Salary will be in the range £3,883 to £6,555

Applications, giving a full curriculum vitae, a copy of the synopsis of their Ph.D , thesis and the names of two academic referees, should be sent to Professor D. G Harnden, Department of Cancer Studies, Medical School, University of Birmingham B15 2TJ 8988(A)

UMIST
LECTURER IN INORGANIC CHEMISTRY

Applications are invited from candidates of either sex for a post of Lecturer in Inorganic Chemistry. The Department of Chemistry has a full teaching programme in Inorganic Chemistry for Honours and Ordinary degrees of the University of Manchester, and has excellent analytical, spectroscopic and synthesis services for research.

Salary will be according to age and experience on the scale £3,883 to £7,754 The appointment will begin as soon as possible.

Application forms and further particulares can be obtained from the Registrar, U M I S T., P.O Box 88, Manchester M60 1QD, or from the Head of Department, Professor P. G Ashmore, and should be returned by February 28, 1979 at the latest.

8939(A)

ASSISTANT PROFESSOR AND ASSOCIATE PROFESSOR

In areas of (1) human genetics (human biochemical genetics or chromosome structure and function) and (2) gene regulation or developmental and molecular genetics of other (lower) eukaryotic organisms Full time positions that involve teaching and independent research. Send curriculum vitae and bibliography to

Dr Stanley N Cohen, Chairman
Department of Genetics
Stanford University Medical Center
Stanford, California 94305.

opportunity employer and we welcome nominations of and applications from women and minority group members

9042(A)

UNIVERSITY OF READING
PROFESSORSHIP OF SOIL SCIENCE

Applications will be sought, p February/March 1979, for the Professorship of Soil Science. Persons wh would be interested in receiving fu ther particulars when they are read are invited to write to the Registra (Room 214, Whiteknights House), Th University, Whiteknights, Readin RG6 2AH. 8942(A)

THE CITY UNIVERSITY
POSTDOCTORAL RESEARCH POSTS IN ORGANIC CHEMISTRY

Applications are invited for the following posts of S.R.C Post-doctoral Research Assistant with Dr S. A Matlin.

(a) A post tenable for a period of two years, on the immobilisation of organo-tin complexes on inorganic supports and studies of their applications to the catalytic reduction of organic compounds

(b) A post tenable for a period of two years, on the synthesis of chiral compounds and their immobilisation on silica, for use in enantiomeric resolutions by H.P.L.C.

Starting salaries will not be lower than £3,883 per annum (under review) plus £450 London Allowance and U.S.S benefits Starting dates will be by mutual agreement.

Applications, including a curriculum vitae and the names of two referees, should be sent as soon as possible to Dr S A Matlin, Department of Chemistry, The City University, Northampton Square, London EC1V 0HB.

9006(A)

ROTHAMSTED EXPERIMENTAL STATION
HARPENDEN, HERTS AL5 2JQ
SCIENTIFIC OFFICER

required for Field Experiments ction Duties include participation the work of the Section, such as eparation and editing publicity aterial describing the general agri- ltural work of the Station, con- cting tours of the Station and monstrations of field experiments r scientists, farmers and members the general public

Qualifications Degree in Agricul- re or other related science, or uivalent qualification plus familiarity th agriculture Driving licence ential.

Appointment is grade of Scientific ficer (£2,839 to £4,415) with starting lary according to qualifications and perience Non-contributory super- nuation

Apply in writing to the Secretary, ving names and addresses of two ferees, and quoting Ref 383 by nuary 31, 1979. Further details on quest 9021(A)

UNIVERSITY COLLEGE LONDON
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANT

To work with Professor P H arke on the isolation of the amidase gulator protein of Pseudomonas ruginosa and to carry out in vitro dies on amidase synthesis

The post is financed for three years the Science Research Council

Applications giving details of quali- ations and the names of two erees should be sent as soon as ssible to Professor P H Clarke, partment of Biochemistry, Univer- y College London, Gower Street, ndon WC1E 6BT 8949(A)

UNIVERSITY OF BRISTOL
EPARTMENT OF PATHOLOGY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from bio- ical scientists with interests in ology, cell biology or immunology. e successful applicant will join a m investigating lymphocyte-virus eractions during EB virus infection vitro The appointment will be for e year in the first instance with e possibility of annual renewal plies, in writing, giving the names l addresses of two referees to Pro- sor M A Epstein, Department of thology, The Medical School, Uni- sity Walk, Bristol BS8 1TD by uary 26, 1979. 8993(A)

OVERSEAS DEVELOPMENT
KNOW-HOW: vital to developing countries

Seed Technologist Malawi

Suitably experienced specialist in seed production techniques and seed certification is required to undertake an assignment as Head of the Seed Technology Unit of the Ministry of Agriculture and Natural Resources The Unit is responsible for the quality control of all certified seed produced in Malawi, and is expected to work in close association with the newly formed National Seed Company Based at Chitedze Research Station, outside the capital Lilongwe, the Unit incorporates a laboratory fully equipped to international standards and is a member of the International Seed Testing Association. The Unit currently has a staff complement of ten. Staff training, the seed Inspectorate in particular, will be an important function of the assignment. The post requires good training capability, experience of research and of developing countries also desirable Background knowledge of tropical cereals and pastures and tobacco an advantage

Appointment 2½ years. Salary (UK taxable) in range £6,300-£9,700 pa, plus tax free overseas allowance in range £1,225-£2,635 pa (Ref 331X).

The post is wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, children's education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, quoting reference stating post concerned, and giving details of age, qualifications and experience to:—

8962(A)

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London SW1E 5DH.

ODM
HELPING NATIONS HELP THEMSELVES

UMIST
RESEARCH ASSISTANT IN CHEMICAL PHYSICS

A Research Assistant, preferably postdoctoral, is required to help with calculations in molecular quantum mechanics, including studies of unusual configurations of small molecules and of a new method of electron population analysis Previous, experience in quantum chemistry and computation would be an advantage Salary in the Scales for Research and Analogous Staffs up to £4,382 according to age and experience The appointment would be for two years, with possible extension for a third year

Enquiries and applications to Professor D W J. Cruickshank, Chemistry Department, U.M.I.S.T., Manchester M60 1QD by January 31, 1979 at latest. 8740(A)

ANALYST TECHNICIAN
(Grade 6)

required for Department of Geology, University of Reading Preference given to applicants experienced in wet chemical methods for silicate analysis, but those with substantial experience in general inorganic analysis by atomic absorption and Auto Analyser techniques also considered Salary according to qualifications and experience in range £3,654 to £4,365 per annum (under review) Further details under Ref. T.N 79A from Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH, to whom applications should be sent to arrive not later than January 26, 1979. 8941(A)

MEDICAL RESEARCH COUNCIL
UNIT ON THE EXPERIMENTAL PATHOLOGY OF SKIN
BIOCHEMIST

required to work on the protein and glycoprotein components of the plasma membranes of normal and pathological epidermal cells.

Applicants should have a Ph.D and a practical knowledge of cell fractionation techniques and membrane biochemistry is essential

Medical Research Council conditions of service. Salary according to age and qualifications

Applications, with the names of two referees, should be sent to the Director, M.R.C Unit on Experimental Pathology of Skin, The Medical School, Birmingham B15 2TJ.

8985(A)

POSTDOCTORATE OR RESEARCH ASSOCIATE IN FISH ECOLOGY

Applications are solicited from individuals with expertise in fish ecology or closely related fields Marine and/or larval fish experience desirable but not essential Successful applicant will coordinate study of interspecific interactions among larval fishes in the St Lawrence estuary. Tenure up to three years Forward curriculum vitae and two letters of reference by February 15, 1979 to:

Dr W C. Leggett, Biology Department, McGill University, 1205 McGregor Avenue, Montreal, P.Q. H3A 1B1. 9009(A)

WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)
DEPARTMENT OF HAEMATOLOGY
RESEARCH OFFICER

A graduate in biochemistry or a related discipline is to be appointed for an initial period of three years to develop techniques of culturing differentiating human erythroid cells using suspension and colony culture techniques The main aim of this work will be to establish factors influencing proliferation, iron metabolism and haem synthesis in normal subjects and patients with haematological disorders

Salary within the range £3,384 to £6,555 (under review) for University Research and Analogous Staff, starting point dependent upon age and qualifications

Applications, which should take the form of a brief curriculum vitae with the names and addresses of two referees, should be sent to the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN (quoting ref No H24/1) by January 22, 1979. The Department may be visited by appointment with Professor A. Jacobs (tel. No 0222/755944, ext. 2375). 9016(A)

Department of Health and Social Security

Pharmacists/ Chemists

The British Pharmacopoeia Commission is responsible for all standards published in Britain for substances and articles used in human and veterinary medicine. The pharmaceutical tests and methods of analysis included in their publications, have largely been evolved in the Pharmacopoeia Laboratory, which is equipped for a range of spectroscopy, chromatography, classical analysis and physical testing.

As a member of a team of scientists, located in Canons Park, Middlesex, the pharmacists/chemists appointed will critically examine proposed specifications for medical substances, and prepare reports for submission to the appropriate committee; maintain quality control measures for medical chemical reference substances used in assays and tests of the British Pharmacopoeia and assist in the development of suitable analytical methods for routine control of pharmaceuticals.

Candidates, normally aged under 27, should already have a good honours degree in chemistry or pharmacy or closely related discipline. Relevant experience is highly desirable, although the job would suit a recently qualified scientist with some experience of laboratory techniques.

Appointment as Scientific Officer (£3110-£4690) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 26 January 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref: SB/13/KD.*

9007(A)

Science Group
CIVIL SERVICE

POSTDOCTORAL BIO-ORGANIC

Isolation and characterisation of cross-linked aromatic peptides. Modification, sequence and biosynthesis of bridges and adjacent residues. Send C. V. and two references to: Dr H. Lipke, Biology Department, University of Massachusetts-Boston, Drochester, MA 02125. An E.O./A.A. employer.

8975(A)

FACULTY POSITION IN EXPERIMENTAL ASTROPHYSICS

The Department of Physics and Astronomy at Northwestern University invites applications for an Assistant Professorship available for September, 1979. This is tenure-track position with an initial appointment for three years. We are seeking a candidate with research experience in observational/experimental astrophysics, and a strong commitment for teaching both astronomy and physics courses. Applications should include a full résumé and the names of at least three references whom can be contacted for letters of recommendation. Please send all applications to: Astrophysics Search Committee, Department of Physics and Astronomy, Northwestern University, Evanston, IL 60201. The closing date for applications will be **March 1, 1979.** Northwestern University is an equal-opportunity/affirmative action employer and welcomes applications from women and members of minority groups.

8978(A)

ASSISTANT PROFESSOR OF ANALYTICAL CHEMISTRY

—tenure track, fall 1979. Send curriculum vitae with a 250-word outline of your research interests. and request 3 letters of recommendation, to Dr D. H. Freeman (Chairman, Search Committee), Department of Chemistry, University of Maryland, College Park, MD 20742 U.S.A. E.O./A.A.E.

8968(A)

THE UNIVERSITY OF MANCHESTER

FACULTY OF MEDICINE

VETERINARY OFFICER

A veterinary graduate with experience in the care and use of laboratory animals is required to manage experimental animal facilities for the Faculty. These will comprise the animal floor of the New Medical School building, extending to about 36,300 sq. feet and an outstation for larger animals and for certain breeding operations. In addition there are separate University-supervised animal facilities at the University Hospital of South Manchester and Hope Hospital, Salford, for which the veterinary officer will be responsible. These facilities cater for all the requirements in the Faculty, including clinical departments. The appointee will be encouraged to pursue his or her own research.

Salary range per annum: £7,508 to £9,263. Superannuation.

Particulars and application forms (returnable by January 15) from the Registrar, The University, Manchester M13 9PL. Quote reference 276/78/N.

8881(A)

NATURAL ENVIRONMENT RESEARCH COUNCIL

DIRECTOR

Institute of Geological Sciences

Applications are invited for the post of Director of the Institute of Geological Sciences, which will become vacant in May 1979 on the retirement of the present Director. The Institute is a constituent body of the Natural Environment Research Council.

It is the primary geological research and advisory body in the United Kingdom, its research covering the earth sciences, including palaeontology, petrography, geophysics, hydrogeology and geochemistry. A considerable proportion of the Institute's work (over 80% currently) is funded by commissions from Government Departments, the EEC and other organisations; work is also carried out for overseas governments and as technical assistance through the Ministry of Overseas Development.

The Institute is in the process of moving many of its functions and staff to a site at Keyworth near Nottingham, where about 650 staff will eventually be based. One of the main concerns for the new Director will be to bring this operation to completion over the next few years and he will establish the Directorate at Keyworth at a suitable time.

The post calls for a geologist of international reputation with a successful record of administration of research and the ability to provide leadership to this large research organisation, which at present has over 1,100 staff.

The post is graded at Under Secretary with a salary from April 1 1979 of £14,714 rising to £16,000 on April 1, 1980. There is a non-contributory superannuation scheme.

Application forms are available from Mr. F. S. Rosier, Natural Environment Research Council, Establishments Division, Polaris House, North Star Avenue, Swindon SN2 1EU (telephone 0793-40101) and should be returned by February 9, 1979.

9035(A)

UNIVERSITY OF GLASGOW MECHAN CHAIR OF AERONAUTICS AND FLUID MECHANICS

Applications are invited for the Mechan Chair of Aeronautics and Fluid Mechanics.

Candidates should have had experience in research, development or design concerned with aircraft or missiles, or their related propulsion, control or navigation systems.

Further particulars may be obtained from the Secretary of the University Court (Room 18), University of Glasgow, Glasgow G12 8QQ, with whom applications (15 copies, or in the case of overseas applicants, one copy), together with the names and addresses of three referees, should be lodged on or before February 15, 1979.

In reply please quote Ref. No. 4336M.

8943(A)

UNIVERSITY OF GLASGOW JAMES WATT CHAIR OF MECHANICAL ENGINEERING

Applications are invited for the James Watt Chair of Mechanical Engineering.

Candidates should have had experience in research, development or design concerned with Engineering Thermodynamics and Heat Transfer and its relation to Fluid Mechanics. This experience could have been in the mechanical, chemical or process engineering fields.

Further particulars may be obtained from the Secretary of the University Court (Room 18), University of Glasgow, Glasgow G12 8QQ, with whom applications (15 copies, or in the case of overseas applicants, one copy), together with the names and addresses of three referees, should be lodged on or before February 28, 1979.

In reply please quote Ref. No. 4337M.

8944(A)

AARHUS UNIVERSITY (DENMARK)

A position as lektor (associate professor with tenure or assistant professor according to qualifications) will be vacant at the Department of Molecular Biology, Aarhus University, Denmark, from June 1, 1979.

A molecular biologist is wanted for the position to work in the field of regulation of expression of essential genes in *E. coli.*

Present permanent staff in the group are Kirsten Gausing, Poul Jørgensen and Niels Ole Kjeldgaard.

The successful applicant must on equal terms with the other members of the group, share the teaching responsibilities — from first-year level to supervision o graduate and postgraduate students It is expected that the successfu applicant eventually will be able to teach in Danish.

Applications containing curriculum vitae, information on scientific and educational activities, a list of publications plus wherever possible 3 copies of published material, and any additional information relevant for the evaluation of the applicant's scientific and educational qualifications should be sent to the Faculty of Science, Aarhus University, Ndr Ringgade 1, DK-8000 Arhus C Denmark before February 15, 1979 The report of the evaluation committee will be sent to all applicants

Further information as regard salary etc. can be obtained from the Department of Molecular Biology, Aarhus University, Universitetsparken, DK-8000 Arhus C Denmark.

8992(A)

UNIVERSITY OF BRISTOL

Applications are invited
for the post of

RESEARCH ASSISTANT/ ASSOCIATE

Within the Department of Anatomy. The post is tenable for up to three years and the appointee will join Professor B T. Pickering's group studying the biosynthesis, ultraneuronal transport and release of neurohypophysical polypeptides. Applicants should have some experience of biochemical, pharmacological, ultrastructural or immunocytochemical techniques; and be in possession of either a Ph.D. or a medical qualification.

Salary will be on the scale £3,883 to £6,555 (under review). Letters of application including curriculum vitae and the names of three referees should be sent to the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TH before January 31, 1979 (quoting ref TLJ) 8896(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(University of London)

Denmark Hill,
London SE5 8RX

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post, which will be supported by a grant from the Cancer Research Campaign to Dr Norman.

The project will involve the use of human lymphoid cell line for the in vitro* investigation of interactions between steroids and other drugs used in the treatment of leukaemia

The appointment will be for three years, with a starting salary of £4,882 per annum plus £450 London Weighting Further particulars may be obtained from the Secretary of the Medical School to whom applications, including full curriculum vitae and naming two referees, should be sent by January 31, 1979. 8989(A)

TECHNICIAN (Grade 4) required in Department of Agriculture and Horticulture, University of Reading, to work on projects, supported by the International Board for Plant Genetic Resources, concerned with the long-term storage of seeds for genetic conservation, under the direction of Professor E. H. Roberts The work involves preparing seeds for low-temperature storage, monitoring deterioration under storage using germination tests and various other physiological techniques H N.C or equivalent qualification desirable. One year appointment in first instance with possibility of renewal. Salary in scale £2,955 to £3,402 per annum (under review). Apply with full details of qualifications and experience, names of 2 referees, and earliest date of starting, quoting Ref TN03A, to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading, Berks. RG6 2AH. 9017(A)

UNIVERSITY OF LONDON KING'S COLLEGE

RESEARCH TECHNICIANS GRADE 5

Two research technicians required in Department of Physics. One to work with Solid State Physics Group. Main responsibilities will be the maintenance, development and setting up of research equipment and preparation of samples. Preference will be given to applicants with experience in either electronics or high vacuum and cryogenic techniques.

The second technician required to work mainly for Infra-Red Spectroscopy and High-Pressure Group. Preference will be given to applicants with experience in spectroscopy or crystal growing and preparation techniques.

The salary for both posts is on scale £3,651 per annum (inclusive) (under review) rising to £4,185. Four weeks holiday annually Contributory pension scheme Apply in writing with full details to The Head Clerk (Ref N210203) King's College London, Strand WC2R 2LS. 9039(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(University of London)

SCHOOL OF PATHOLOGY

RESEARCH IN CHEMICAL CARCINOGENESIS

Applications are invited from recent graduates for the post of Research Assistant to work on the mechanism of malignant transformation of brain cells by nitrosamides Cell culture techniques are used extensively in this project. A background in Microbiology, Biochemistry or Molecular Biology would be advantageous The post, funded by the Cancer Research Campaign, is available immediately, and potentially tenable up to December 31, 1981 Starting salary £3,858 per annum including London Allowance

Applications including a curriculum vitae and the names of two referees to, Dr J P. Roscoe, (N) The Middlesex Hospital Medical School, School of Pathology, Riding House Street, London W1P 7LD 9033(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

SUB-DEPARTMENT OF MOLECULAR BIOPHYSICS

RESEARCH ASSISTANT

Applications are invited for the above post, financed by the World Health Organisation, to work on peptide secretion from human tumour cells Experience of tissue culture techniques and/or radioimmunoassay desirable but not essential

Salary within the range £3,858 to £5,340 per annum (including London Allowance) according to age and experience.

Applications, including a curriculum vitae and the names of two referees should be sent to The Secretary, (N) Sub-Department of Molecular Biophysics, Department of Nuclear Medicine, The Middlesex Hospital Medical School, London W1N 8AA by January 26, 1979. 9032(A)

AGRICULTURAL RESEARCH COUNCIL FOOD RESEARCH INSTITUTE

Applications are invited for the following posts which have arisen from the recent formation of a Nutrition and Food Quality Division.

(1) PHYSIOLOGIST

Nutrient Interactions Group
(Senior Scientific Officer/Principal Scientific Officer Grade)

First or Upper Second Class Honours degree in Physiology with at least four years' relevant postgraduate experience, including studies on intestinal absorption, and preferably a Ph D. Quote ref: 77/3.

(2) NUTRITIONIST

Nutrient Interactions Group
(Higher Scientific Officer/Senior Scientific Officer Grade)

First or Upper Second Class Honours degree in Nutrition or Physiology/Nutrition with at least 2 years' post-graduate experience, including nutritional studies with small animals. Quote ref: 75/8.

(3) ASSISTANT NUTRITIONIST

Nutrient Interactions Group
(Scientific Officer Grade)

Degree or HNC, HND or equivalent qualification in nutrition or related subject. Quote ref: 77/2

(4) NUTRITIONIST

Nutritional Composition Group.
(Higher Scientific Officer/Senior Scientific Officer Grade)

First or Upper Second Class Honours degree in Nutrition or closely related subject with at least 2 years' post-graduate experience in connection with the analysis of foods or assessment of their nutritional value. Quote ref. 77/8.

(5) SENIOR LABORATORY ANIMAL TECHNICIAN

(Scientific Officer Grade)

Associateship of the Institute of Animal Technicians or equivalent qualification and several years' post-qualifying experience required. Quote ref: 75/12.

Salary Scales:

Principal Scientific Officer ..	**£6,609 — £8,461**
Senior Scientific Officer .	**£5,164 — £6,898**
Higher Scientific Officer .	**£4,101 — £5,448**
Scientific Officer	**£2,839 — £4,415**

Non-contributory superannuation scheme at age 18 years and over. Five day week and flexible working hours scheme operated

Application form and further particulars from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA. Closing Date: 19th January, 1979. 8948(A)

NEW ZEALAND UNIVERSITY OF CANTERBURY

Christchurch

Applications are invited for the following positions

SENIOR LECTURER/ LECTURER IN COMPUTER SCIENCE

Applicants should have interests within the (very broad) areas of computer systems and languages, theory of computing, information systems, or other areas of non-numerical applications. Preference may be given to an applicant qualified in data base techniques, information systems and related areas.

The salary for Lecturers is on a scale from NZ$11,894 to NZ$14,488 per annum. Promotion may be made on academic merit to Senior Lecturer where the salary range is from NZ$14,983 to NZ$17,145 (bar), NZ$18,380 per annum.

Applications close on *February 28, 1979.*

Particulars, including information on travel and removal allowances, study leave, housing and superannuation, may be obtained from the Association of Commonwealth Universities (Appts), 36, Gordon Square, London WC1H 0PF. 9043(K)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF EARTH SCIENCES

Applications are invited for a post of

POSTDOCTORAL RESEARCH FELLOW

for work on palaeomagnetic studies of metamorphic cooling in the Scottish Caledonides on a project financed by the Natural Environment Research Council. The appointment will be made for a fixed term of three years and the successful candidate will work under the direction of Professor J. C. Briden. It is hoped to make an appointment with effect from April 1, 1978 or as soon as possible thereafter.

Salary in the range £3,883 to £4,382 on the IA scale Research and Analogous Staff (£3,883 to £6,555) (under review).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 49/10D Closing date for applications February 19, 1979. 9027(A)

The International Atomic Energy Agency

seeks for its laboratory at Monaco a Senior Marine Alpha Radioactivity Specialist. An advanced University degree in chemistry, physics or chemical oceanography and 10 years' experience in polonium and plutonium techniques are essential. Initial contract two years. Emoluments depending on experience US$35,000 per annum tax-free. Additional allowances if married. Travel and removal expenses paid. Six weeks annual leave. Send curriculum vitae under VN 61/78 to IAEA, Division of Personnel, P.O. Box 590, A-1011 Vienna, Austria. 8976(A)

UNIVERSITY OF SURINAME

Faculty of Natural Resources
Department of Geology and Mining

TEACHING POSTS IN GEOLOGY AND MINING

GENERAL: The Department is a part of the recently established Faculty of Natural Resources. The course now offered is of B.Sc. level. It is, however, likely that in the near future graduate student will be admitted.

The period of first contract for non-Surinamese staff members is three years, whereupon the contract may be extended. Annual salary is the equivalent of US $11,000 (under review), the contract bonus 25% thereof. On first appointment and on termination the University will pay the air passage and a baggage allowance.

Applicants for these posts should hold the degree of M.Sc. or equivalent with considerable teaching and research experience preferably in the tropics. Possession of a Ph.D. is an advantage, just as management experience.

DUTIES WILL INCLUDE:

—teaching (a wide range) of geology and mining subjects;

—to evaluate, adjust and develop the programme of study;

—participation in the research programme of the faculty;

—to carry out organisational activities.

For information please write to the Board of the Faculty of Natural Resources, P.O.B. 1914, Paramaribo, Suriname (S.Am.). 8998(A)

LOTHIAN HEALTH BOARD
PRINCIPAL GRADE CYTOGENETICIST

Applications are invited for the above post based at the Department of Pathology, Royal Hospital for Sick Children, Edinburgh.

Candidates must be graduates with considerable experience in all aspects of cytogenetic theory and practice. A higher degree would be considered essential The successful applicant would be expected to partake in medical undergraduate teaching. The Department is responsible for practical cytogenetic diagnosis on a supra-area basis. Consideration would be given to offering the successful candidate honorary University status.

Arrangements to visit the unit may be made by telephoning 031-667 1991, ext 270.

Applications which should be typewritten, giving particulars of age, qualifications and previous experience, together with the names and addresses of two referees should be lodged with the Secretary, 11 Drumsheugh Gardens, Edinburgh EH3 7QQ within two weeks from the date of this advertisement.

9025(A)

COUNTY OF SOUTH GLAMORGAN

INSTITUTE OF HIGHER EDUCATION

Faculty of Science

DEPARTMENT OF SCIENCE

Applications are invited for the following full-time appointment commencing in February 1979. The appointment will be in a temporary capacity because of the absence on maternity leave of the present holder of the post The approximate date of termination will be in July, 1979.

SENIOR LECTURER IN NUTRITION and PHYSIOLOGY

Salary: £6,051 to £7,572

Applicants should be graduates with appropriate teaching experience The person appointed will be involved in the teaching of Nutrition to diploma and undergraduate dietetics students and to students studying the Food Science Option of an H N D Course in Applied Biology Physiology will be taught to students attending Dietetics and Nursing Diploma Courses.

Application forms and further particulars are obtainable from the Deputy Principal, South Glamorgan Institute of Higher Education, Llandaff Centre, Western Avenue, Cardiff CF5 2YB, to whom they should be returned within 10 DAYS of the appearance of this advertisement.

F. J. Adamas, Director of Education, Education Offices, Kingsway, Cardiff. 8946(A)

BEDFORD COLLEGE
(University of London)
SAFETY/RADIATION PROTECTION OFFICER

Applications are invited for the above new post for an officer with a scientific/technical bias to work under the direction of the College Secretary in ensuring compliance with statutory requirements particularly with regard to radiological protection Previous practical experience of radioisotope and/or radiation work essential Initial salary within gross range £3,834 to £7,005 p a Further particulars from the undersigned to whom applications must be submitted by February 22, 1979

Senior Assistant Secretary N (Personnel), Bedford College, Regent's Park, London NW1 4NS, Tel 01-486 4400, Ext 312 9037(A)

THE UNIVERSITY OF WEST INDIES—TRINIDAD

Applications are invited for the vacant temporary post of:

SENIOR LECTURER/ LECTURER/ASSISTANT LECTURER

in the

DEPARTMENT OF LIVESTOCK SCIENCE, FACULTY OF AGRICULTURE

Preference will be given to applicants holding a first degree in animal science or veterinary medicine with postgraduate qualifications in animal physiology Interest and experience in the physiology of dairy cattle reproduction (infertility) and/or adaptation of ruminants to hot, humid climates would be an advantage The appointment will be for 2 years with the possibility of extension for a total of 5 years

Salary Scales (1977-78 under review) Senior Lecturer, TT$25,827 to 34,017 per annum, Lecturer TT$19,071 to 29,799 per annum; Assistant Lecturer TT$15,480 to 16,974 per annum (£1 sterling=TT$4 74) F S S U Unfurnished accommodation if available at 10% or furnished at 12½% or housing allowance of 20% of pensionable salary Family passages. Study and Travel Grant

Detailed application (2 copies) with curriculum vitae and naming three referees to be sent direct to Secretary, U W I, St Augustine, Trinidad, as soon as possible Applicants resident in the U K should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address. 8956(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appoin ment to the following.

Research School of Earth Sciences

ENVIRONMENTAL GEOCHEMISTRY

RESEARCH FELLOWS
(2 posts)

The School is proposing to con mence work in Environmental Ge chemistry and invites application from appropriately qualified eart scientists This is an interdisciplina field embracing atmospheric chemistr chemical oceanography and palae climatology, and involves the study the chemical interactions between t atmosphere, hydrosphere and sol earth

Initially the appointees will attached to the isotope group head by Dr W Compston, where ma spectrometric facilities for stable a radiogenic isotope geochemistry a available While specific research topi have not yet been chosen, it is expe ted that studies will be orient towards the carbon cycle and palae climatology in the southern hemisphe using C, O isotopic analyses.

Exceptionally well qualified cand dates may be considered for appoin ment at Senior Research Fellow lev

CLOSING DATE. **MARCH 3 1979.**

Salary on appointment will be accordance with qualifications and e perience within the ranges Seni Research Fellow $A21,201 to $25,2 per annum, Research Fellow $A15,1 to $19,813 per annum Current e change rates $A1=$US1 14 · £0 58

Tenure: Appointment as Seni Research Fellow/Research Fellow normally for 3 years in the fi instance with the possibility of ext sion to 5 years

Reasonable appointment expenses a paid Superannuation benefits a available for applicants who a eligible to contribute Assistance w housing is provided for an appoint from outside Canberra

The University reserves the ri not to make an appointment or make an appointment by invitation any time.

Prospective applicants should obt further particulars from the Asso tion of Commonwealth Universit (Appts.), 36 Gordon Square, Lond WC1H 0PF. 8955(A)

UNIVERSITY OF CAMBRIDGE
UNIVERSITY LECTURER or ASSISTANT LECTURER
in CHEMICAL ENGINEERING

Applications are invited for the post of University Lecturer or Assistant Lecturer in Chemical Engineering. The successful applicant will take part in the teaching programme in the Department, and is also expected to undertake research in a field of his or her own choice. Graduates in chemical engineering would be particularly appropriate candidates, but applications will also be welcomed from graduates in other branches of engineering, or in chemistry, biochemistry or physics. Industrial experience would be an advantage but is not essential.

For a Lecturer, the initial appointment is for three years with the possibility of re-appointment to the retiring age. The present pensionable scale of stipends for a University Lecturer is £5,367 a year, rising by 12 annual increments to **£8,257.**

For an Assistant Lecturer the initial appointment is for three years with the possibility of re-appointment for two further years. The present pensionable scale of stipends for a University Assistant Lecturer is £4,133 a year, rising by four annual increments to £5,129 a year.

Further information may be obtained from Professor J. F. Davidson, Department of Chemical Engineering, Pembroke Street, Cambridge CB2 3RA. Closing date February 28, 1979. 9026(A)

ANIMAL HEALTH TRUST
SMALL ANIMALS CENTRE
SENIOR
RESEARCH ASSISTANT

We are expanding our renal research
ıit and wish to appoint a senior re-
arch assistant with suitable experi-
ıce in applied Biochemistry to assist
the investigation of canine chronic
nal disease. The successful candidate
ill be expected to take an active
terest in the project as a whole and
apply a variety of separation tech-
ques to the study of urine proteins
d "uraemic toxins". This five year
llaborative study is supported by the
ellcome Trust. The initial appoint-
ent will be for a three year period.
arting salary will be in the range
,032 to £5,697 (with F.S.S.U./U.S.S.)
cording to age and experience.

Technical staff with suitable experi-
ıce are invited to apply.

The laboratories are modern, well
uipped and situated four miles from
ewmarket, in pleasant park land.

For further information please con-
ct Dr D. F. Macdougall at The Ani-
al Health Trust, Small Animals
entre, Lanwades Park. Kennett, Nr.
ewmarket, Suffolk, CB8 7PN. (Tel:
38—750543).

Applications, to include full curri-
ılum vitae and the names of two
ferees, to the Director, by February
1979. 9030(A)

ASSISTANT PROFESSOR

The Division of Tumor Immunology
seeking a new faculty member at
e assisting professor level. We are
eking an investigator with a strong
terest and previous experience in
ıalysing and characterising the hu-
an cellular immune response. Wo-
en and minority candidates are
ıcouraged to apply. Please send cur-
culum vitae and the names of three
ferences to: Search Committee, Divi-
on of Tumor Immunology, Sidney
ırber Cancer Institute, 44 Binney
reet, Boston, MA 02115. An Equal
pportunity/Affirmative Action Em-
oyer. 9029(A)

EUROPEAN MOLECULAR BIOLOGY LABORATORY
JUNIOR BIOCHEMIST

Applications are invited for the post
Junior Biochemist at EMBL's
ıtstation at **Grenoble, France.**

The successful applicant will join a
search project on aspects of muscle
ochemistry, in particular the deter-
ination of the amino-acid sequence
the rod portion of the myosin
olecule. Information on this
quence will be used as primary data
r physical structural studies involv-
g such techniques as electron
icroscopy, X-ray and neutron
ffraction.

A Ph.D. qualification, followed by
me experience in an appropriate
ld, is required.

The salary offered will be **FF 6643**
onthly, after deduction of tax.
rtain allowances are payable in
dition, depending on personal
rcumstances.

Applications, quoting **reference 79/1,**
th curriculum vitae and a brief
atement of interests, together with
mes of referees, to:—

Sir John Kendrew, Director General,
MBL, Postfach 10.2209, D-6900
idelberg, Germany. 9040(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
TECHNICIAN 4

Required in Biochemistry Department to work on a 2 year C.R.C. project involving virus-cell interactions. Duties will include preparing specimens for electron microscopy and photography. Appropriate laboratory experience essential. HNC or degree desirable. Application forms available from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE. 9028(A)

UNIVERSITY OF LONDON
KING'S COLLEGE
RESEARCH TECHNICIAN GRADE 3

required to assist with project investigating rabbit red blood cell development, with particular reference to MRNA and protein synthesis. Knowledge of protein fractionation chromatography, centrifugation and radioactive techniques is desirable. Minimum qualifications O.N.C./H.N.C. or equivalent and three years relevant experience. This post is financed by the M.R.C. until May 31, 1981. Salary on scale £3,153 per annum rising to £3,525 per annum inclusive (under review). Four weeks annual holiday. College contributory pension scheme. Apply in writing with full details to: The Head Clerk (Ref. (N) 210206), King's College London, Strand, WC2R 2LS. 9034(A)

QUEEN MARY COLLEGE
University of London
MILLIMETRE WAVE/ MOLECULAR LINE ASTRONOMY

Applications are invited for a

POSTDOCTORAL RESEARCH ASSOCIATESHIP

for work with the Millimetre Wave Astronomy Group, whose research activities involve observation and interpretation of emission from molecules in galactic HII regions and molecular clouds. The observations are made using heterodyne techniques giving high spectral resolution, from high altitude observatories in the northern and southern hemispheres. Suitable candidates would have a strong interpretational background in astronomy, or a relevant field of physical science, but applicants with more experimental experience are not excluded. The job will entail assistance in the planning and execution of the group's observational programme.

Appointment for three years from as soon as possible. Initial salary in range (under review) £3,883 to £4,382 per annum plus £450 London Allowance.

Further details obtainable from Dr J. E. Beckman (N), Physics Department, Queen Mary College, Mile End Road, London E1 4NS.

8953(A)

FELLOWSHIPS

UNIVERSITY OF WARWICK
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a one year Postdoctoral Research Fellowship in the Department of Chemistry and Molecular Sciences to work on the photochemistry of metal complexes in the solid state with Drs N. W. Alcock and T. J. Kemp. The project will involve synthesis, photochemistry and X-ray diffraction and would be suitable for someone with expertise in one of these fields (particularly the first) wishing to extend their range of skills. The post is available immediately. Initial salary will be on one of the first three points of the Research Range IA scale: £3,883 to (£4,382) to £6,555 per annum (under review). Applications (including a detailed curriculum vitae and the names of two referees) to the Academic Registrar, University of Warwick, Coventry CV4 7AL, as soon as possible. Please quote Ref. No: 18/A/78. 8957(E)

IMPERIAL CANCER RESEARCH FUND
London, England
VISITING FELLOWSHIPS

The Council of the Fund invites applications for Visiting Fellowships to be held in any of the following: the Central Laboratories of the Fund at Lincoln's Inn Fields; Mill Hill Laboratories; the Breast Cancer Unit, Guy's Hospital; Cancer Epidemiology and Clinical Trials Unit, Oxford University; Medical Oncology Unit, St Bartholomew's Hospital; or Tumour Immunology Unit, University College, London.

These Fellowships will be open to any applicant, irrespective of citizenship who has (or is about to obtain) a Ph.D. or its equivalent and who is temporarily or permanently resident outside the UK. Remuneration will be comparable with postdoctoral fellowships in country of origin; the award will be for one year. Return air fares at the lowest practicable rates will be paid for the Fellow and family, and medical insurance where necessary. The award constitutes a Contract of Service namely to undertake specified cancer studies for a stated period. Fellowships are not pensionable.

Application to the Director of Research, Imperial Cancer Research Fund, Lincoln's Inn Fields, London W.C.2, England, should be accompanied by full C.V. and list of publications, the names of two referees, an account of the applicant's current and proposed research extending to about 500 words, and a short account of the relevance of the above work to the alleviation of cancer. The laboratory or unit in which the candidate wishes to work should be specified. Closing date March 31, 1979. 9020(E)

UNIVERSITY OF SALFORD
DEPARTMENT OF CHEMISTRY AND APPLIED CHEMISTRY

Applications are invited for the following research fellowships in organic Chemistry, sponsored by the Science Research Council;

(1) Synthesis of prostanoids containing a thiophen ring, under the supervision of Dr O. Meth-Cohn and Dr S. M. Roberts, commencing April 1, 1979 or soon as possible thereafter (Ref: CH/248/N).

(2) The Chemistry of enamidines, under the supervision of Dr B. J. Wakefield, commencing June 1, 1979 or as soon as possible thereafter (Ref: CH/249/N).

Commencing salary within the range: £3,883 to £4,382 per annum; U.S.S.

Application forms are available from the Registrar, University of Salford, Salford M5 4WT (Tel: 061-736 5843, Ext. 215) to whom completed applications should be returned by February 28, 1979 for post (1) and March 31, 1979 for post (2). Please quote appropriate reference. 9012(E)

UNIVERSITY OF ABERDEEN
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for an S.R.C. Research Fellowship working with Dr A. R. West on novel solid state energy storage systems.

Appointment will be from April 1, [illegible], or as soon thereafter as possible, and will be tenable for two years.

Salary £3,883 to £4,382 per annum on Research and Analogous Staff Range 1A Scale.

Further particulars from The Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by January 26, 1979.

9036(E)

MAGDALEN COLLEGE, OXFORD
FELLOWSHIP IN PURE MATHEMATICS

The College proposes to elect with effect from October 1, 1979 a Fellow and Tutor (male or female) in Pure Mathematics. A part-time University Lectureship is associated with the Fellowship and the successful candidate will be eligible for appointment to this post.

Completed applications, together with the names of three referees, should be submitted by February 12, 1979. Application forms and further information can be obtained from the President, Magdalen College, Oxford.

9038(E)

BIRKBECK COLLEGE
(University of London)
DEPARTMENT OF CHEMISTRY
RESEARCH OFFICER IN ORGANIC CHEMISTRY

Applications are invited for a postdoctoral fellowship to initiate a project on the synthesis of acetylenic derivatives of carbohydrates of potential biological importance. The appointment will be for one year initially, but is likely to be renewed for a second year. Salary in the range £3,685 to £4,155 (under review from October 1, 1978) plus £450 London Allowance. Applications in writing with curriculum vitae and the names of two referees to Dr N. R. Williams, Birkbeck College, Department of Chemistry, Malet Street, London WC1E 7HX.

8981(E)

COURSES

ELECTROMAGNETIC SCATTERING AND PARTICULATES

A post experience course will be held at Imperial College from March 26 to 28, 1979. Details from Dr A. R. Jones, Chem. Eng., and Chem. Tech., Imperial College, London SW7 2BY. Tel: 01-589 5111, Ext. 1906. 8980(D)

ANNOUNCEMENTS

ANNOUNCEMENT

The fourth annual "Advanced Institute in Methods of Immunological Diagnosis", conducted under the auspices of the World Health Organization Collaborating Laboratory for the Serology of Auto-immune Diseases at Wayne State University School of Medicine, will be held June 17-23, 1979. The course is to be held on the wooded campus of Cranbrook Educational Community in Bloomfield Hills, Michigan.

This year the course will emphasize selected, newer methods applicable to the diagnosis of human diseases.

Category I credit for post graduate medical education has been approved. Deadline for receiving applications is April 15. Further information can be obtained by writing to:

Noel R. Rose, M.D., Ph.D.
Department of Immunology and Microbiology
Wayne State University School of Medicine
540 East Canfield
Detroit, Michigan 48201

8838(G)

MISCELLANEOUS

CHINA: SCIENCE AND SOCIETY

SACU's 21 day Science Study Tour leaves March 18: an unusual opportunity to see China and study the rapidly-changing role of science and technology in Chinese society. Assess China's achievements in advanced research, the popularisation of science, and the use of 'appropriate' technology.

CHUNGKING AND THE YANGSTE GORGES

Specialist tour in August with varied coverage for Geographers and Environmentalists, visiting Peking, Wuhan, Canton and Szechuan province, whose mountains enclose the famous Red Basin, and three-day investigative trip through the Yangste Gorges.

Full preparatory study programme.

Further details: Society for Anglo-Chinese Understanding, 152 Camden High Street, London NW1 0NE. Tel. 01-267 9841. 8996(J)

STUDENTSHIPS

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

JOHN LUCAS WALKER SENIOR STUDENTSHIP

The Managers invite applications for a John Lucas Walker Senior Studentship, the holder of which must devote himself/herself to original research in any of the following branches of Pathology (Cellular Pathology, Immunology, Microbiology and Virology). Applicants should already have completed a period of postgraduate training (Ph.D. or equivalent). Applications should be sent not later than February 19, 1979 to Professor P. Wildy, Department of Pathology, Tennis Court Road, Cambridge. The studentship is tenable for 3 years; its annual value will accord with the scales laid down for University Research Staff. 9031(F)

UNIVERSITY HOSPITAL

Saskatoon, Saskatchewan
S7N 0W8 Canada

Three-year Studentship leading to an M.Sc./Ph.D. in multi-disciplinary areas of biological psychiatry (neurochemistry, clinical chemistry, neuropharmacology) are available. Applications are invited from graduates with a first or upper second class honours or equivalent qualifications in a suitable biological subject to read for a higher degree. Interested persons should write to Dr A. V. Juorio, Head Basic Studies, Psychiatric Research Division, University Hospital, Saskatoon, Saskatchewan, S7N 0W8, Canada, including a curriculum vitae and the name and addresses of two referees. Non-Canadian applicants are advised to write before February 28, 1979, and landed immigrants to Canada and Canadian nationals before July 31, 1979. 8977(F)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF FIBRE SCIENCE

RESEARCH STUDENTSHIPS

Applications are invited from suitably qualified science or engineering graduates for two studentships. One for work on the handle and texture of fabrics would suit a candidate with a physics/mathematics background. The other dealing with fibre structure/property relationships would suit a chemist or materials scientist.

S.R.C. scales will apply and successful applicants will be expected to register for a higher degree.

Further information and application forms can be obtained from Dr H. M. Elder, Chairman of Department, Department of Fibre Science, University of Strathclyde, George Street, Glasgow. 8999(F)

ST JOHN'S COLLEGE CAMBRIDGE RESEARCH STUDENTSHIPS

The College invites applications for a number of Research Studentships, normally tenable for three years and of such value as will, in addition to the payment of fees, bring the student's total emoluments to £1,710 a year, for men who are not already members of the College but who propose to register as Ph.D. students at the University. Candidates should have gained or have a strong prospect of gaining a first-class honours degree or equivalent.

Further details and application forms can be obtained from the Senior Tutor, St John's College, Cambridge CB2 1TP. Completed applcations must be received by April 1, 1979. 8986(F)

ASSISTANTSHIPS

UNIVERSITY OF SOUTHAMPTON

SCHOOL OF BIOCHEMICAL AND PHYSIOLOGICAL SCIENCES

GRADUATE RESEARCH ASSISTANTSHIP

Applications are invited for appointment as a GRADUATE Research Assistant in the BIOCHEMISTRY GROUP of this School, to work with Dr D. P. Bloxham on the applications of thiosulphonate reagents to problems in protein structure and function. Applicants should have a good honours degree in biochemistry or chemistry and should have an interest in enzymology. The appointment, which is S.R.C. supported, will be made as soon as possible and is initially for one year, renewable for a further two years.

The salary will be within £3,884 to £4,883 per annum (under review) on Scale 1B for Research and Analogous Staff; U.S.S. benefits.

Applications giving date of birth, curriculum vitae and the names and addresses of two referees, should be sent to Mrs P. Vaughan-Smith, The University, Southampton SO9 5NH, by January 20, 1979, quoting reference 1043/R/Na. 8889(P)

UNIVERSITY OF CALGARY GRADUATE ASSISTANTSHIPS (Teaching and Research)

Available for students proceeding to the M.Sc. and Ph.D. degrees in all branches of CHEMISTRY and BIOCHEMISTRY. **The** assistantships are valued at $7,000 to $8,000 p.a. and include remission of program fees. Travel allowance also available.

For further information write to:

Department of Chemistry
Chairman of Graduate Studies
UNIVERSITY OF CALGARY
Calgary, Alberta, Canada
T2N 1N4

Please indicate field of interest. 8969(P)

UNIVERSITY OF DUNDEE

DEPARTMENT OF MATHEMATICS

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANTSHIP

to work with Dr D. F. Griffiths in the above Department on finite element methods in fluid dynamics. The appointment will be for two years at a starting salary in the range £3,883 to £4,382 depending on age and qualifications.

Applications, which should include a curriculum vitae and the names and addresses of two referees, should be sent by February 13, 1979 to The Secretary, The University, Dundee DD1 4HN, from whom further particulars may be obtained. Please quote Reference EST/73/78N). 9015(P)

LECTURES

1st FEBS LECTURE TOUR

A new Lectureship Programme has been started by the Advanced Courses Committee of the Federation of European Biochemical Societies. The 1st FEBS Lecture Tour will take place in 1979.

GARY FELSENFELD

(Bethesda, Md)

will lecture on

"Nucleosome Architecture and Chromatin Function"

as follows:

February 1,	London (King's College Department of Biophysics)
February 1,	Cambridge (M.R.C. Laboratory of Molecular Biology)
February 5,	Orléans (Centre de Biophysique Moléculaire)
February 6,	Paris (Institut de Recherche en Biologie Moléculaire)
February 8,	Munich (Institut für Physiologische Chemie, Physikalische Biochemie, und Zellbiologie)
February 9,	Heidelberg (European Molecular Biology Laboratory)
February 12,	Strasbourg (Laboratoire de Génétique Moléculaire des Eucaryotes)
February 14,	Barcelona (Depto. de Química Macromolecular)

8979(K)

A NATO SCHOOL ON TOPICS IN THEORETICAL ORGANIC CHEMISTRY

will be held in Gargnano (Garda Lake), Italy on June 18-30, 1979

Lectures will be delivered by:

A Bernardi (Italy)
E. Clementi (Italy)
I. G. Csizmadia (Canada)
N. Epiotis (U.S.A.)
P. von R. Schleyer (Germany)

Intending participants should contact: Professor A. Mangini, NATO School, Istituto Chimica Organica, Università, Viale Risorgimento 4, 40136 Bolonga, Italy. 8971(K)

GRANTS/SCHOLARSHIPS

MULTIPLE SCLEROSIS RESEARCH

Applications are invited for Grants funded by the Society for research related to Multiple Sclerosis. Support will be provided for approved clinical or epidemiological work as well as for laboratory-based studies within Australia.

Applications from suitably qualified persons will also be considered for Post-Doctoral Fellowships. Intending applicants for project grants should write for further information to the Executive Director, National Multiple Sclerosis Society of Australia, 616 Riversdale Road, Camberwell, 3124, Australia. Applications close on Saturday, March 31, 1979.

Professor D. R. Curtis, Chairman, Research Advisory Board, National Multiple Sclerosis of Australia. 8984(H)

TH7 ROYAL SOCIETY THE RUTHERFORD SCHOLARSHIP

This award, for graduates of e ceptional promise and ability who a under 26 years of age on May 1979, is for experimental research any of the natural sciences, with preference for experimental physics there are candidates of similar mer It will be tenable for three yeai from October 1, 1979 or such oth date as may be arranged, in son part of the British Commonweal other than that in which the applica graduated. The value, adjusted to me the circumstances, will be £2,250 p annum on appointment (under revie if held in the United Kingdom, wi allowances for travel, university fe etc. It will not normally be award to a graduate who has already held senior research award.

Applications should be made follows:

From university graduates *in t United Kingdom:* before February 1 1979 on forms obtainable from t Executive Secretary, The Roy Society, 6 Carlton House Terrac London SW1Y 5AG.

From university graduates *outsi the United Kingdom:* through th universities to the Royal Commissi for the Exhibition of 1851 on forr of application for the Commissione Science Research Scholarship to obtained from the registrars administrative authorities of th universities. 8967(H)

IMPERIAL CANCER RESEARCH FUND BURSARIES FOR TRAINING IN RESEARCH

A small number of Bursaries will be awarded at the laboratories in Lincoln's Inn Fields and Mill Hill for full-time studies leading to higher degrees in research fields relevant to cancer, that include chemistry, cell and molecular biology.

The awards will be tenable for three years from October 1, 1979 with a non-pensionable grant of £2,952 a year (subject to tax) and, in some cases, additional allowances.

Applications are invited from British subjects normally resident in the U.K. and not over 25 years of age, who hope to obtain First or Upper Second class Honours Degrees in Science in 1979.

Further details and application forms available from

The Personnel Officer
Imperial Cancer Research Fund
Lincoln's Inn Fields
London WC2A 3PX

Completed application forms should reach the Director of Research at the above address not later than February 14, 1979. 9019(H)

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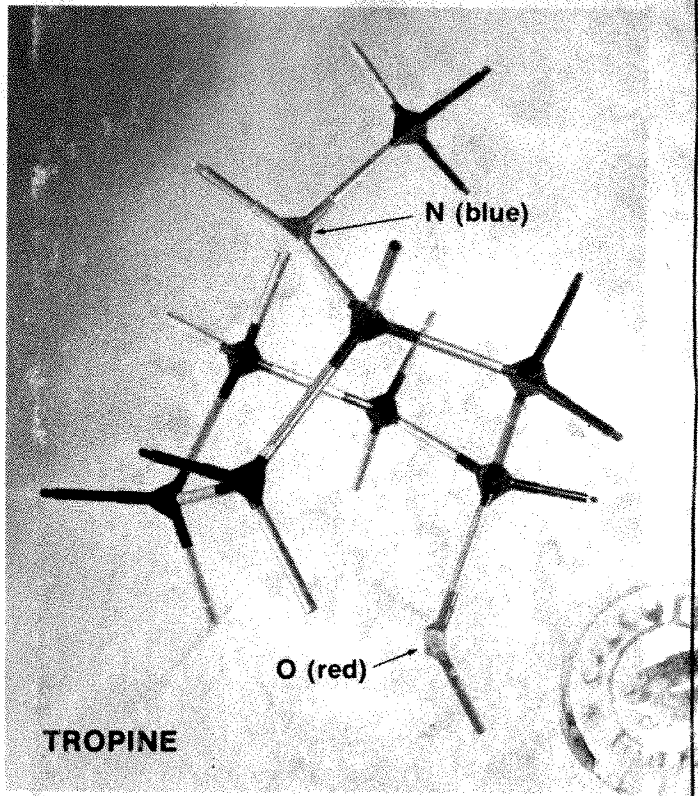

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References:
(1) L.F. Fieser, *J. Chem. Ed.*, **40**, 62 (1963).
(2) L.F. Fieser, *ibid.*, **40**, 457 (1963).
(3) L.F. Fieser, *ibid.*, **42**, 408 (1965).
(4) L.F. Fieser, "Chemistry in Three Dimensions," Aldrich Catalog Number Z10,160-5.

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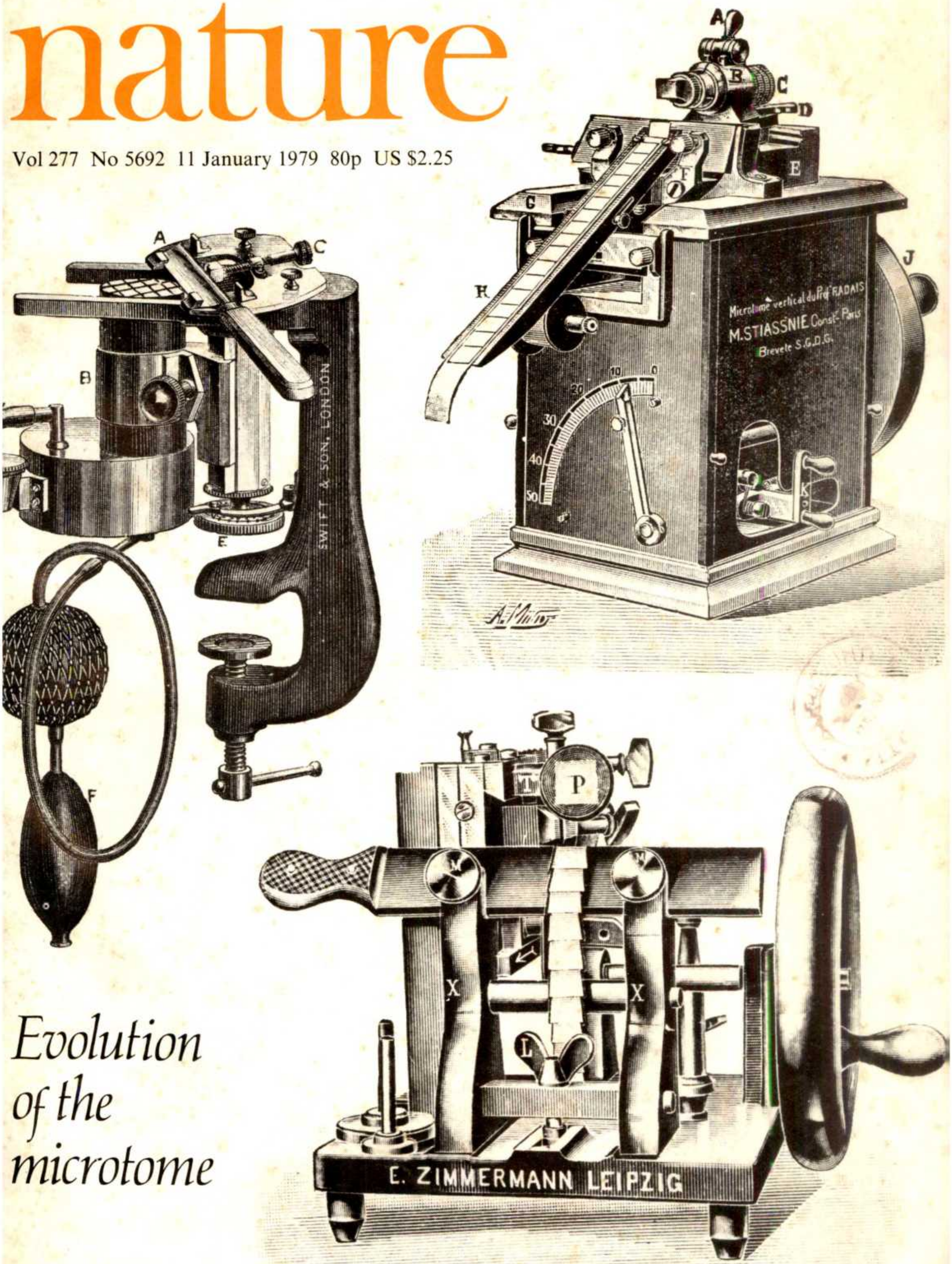

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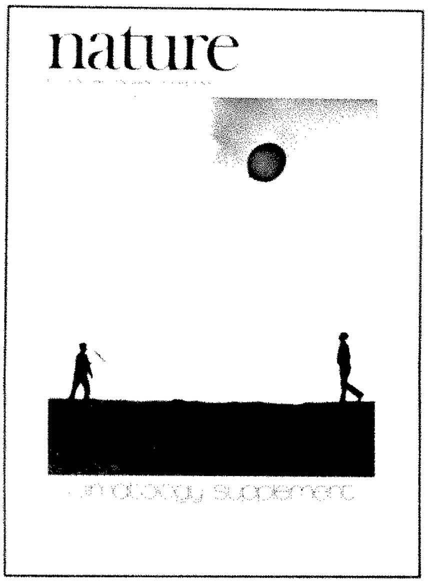

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Cover picture

Left, Swift's microtome (1882); *top right,* the Radais design (1904); *bottom right,* Zimmermann–Minot microtome (1908). All taken from *A History of Microtechnique,* reviewed on page 155.

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Smallpox: ignorance is never bliss

IGNORANCE confronts ignorance in the case of the release of the smallpox virus, *Variola major* Abid, from a Birmingham University laboratory last August. Clive Jenkins, leader of the Association of Scientific, Technical and Managerial Staffs (ASTMS), a trades union taking an increasing interest in laboratory safety in the UK, 'published' the confidential Shooter Report on the Birmingham affair last week. This has removed our ignorance of the events which led up to the smallpox release, as we describe in the next few pages; but it has not removed our ignorance of the smallpox virus, whose relationships among its many strains and to the various animal poxes—from which it might re-emerge—remains obscure. Professor Henry Bedson was working on the protein characterisation of the various pox viruses, under pressure from the forthcoming closure of his laboratory, when the escape occurred. The World Health Organisation (WHO) team, whose work has led to the virtual eradication of *Variola major* as a cause of disease, had earlier in the year described his work as "fascinating", but within a few weeks Professor Bedson had committed suicide.

Professor Bedson, the Shooter Report implies, had been guilty of his own kind of ignorance: wilfully or otherwise he had ignored elementary safety precautions in the handling of the virus. According to one member of the Dangerous Pathogens Advisory Group (DPAG), which licensed Bedson's laboratory as adequate in 1976, the equipment Bedson was using was unsafe "except for the mildest pathogens". Interestingly enough, the first this DPAG member had seen of the apparatus was on television news last week.

None of these various kinds of ignorance is tolerable. First we must know more of the smallpox virus and its relatives. Second, those working with pathogenic—or potentially pathogenic—organisms must be prepared to educate themselves about the mechanisms by which those organisms can escape. (This involves as much physics as biology). And third, after DPAG is disbanded (it must be, if only to restore public confidence), its successor must be provided with sufficient critical investigative power that its members do not have to rely on the television for their information.

Most of what needs to be done follows from these three requirements. First, Professor Dumbell, whose laboratory at St Mary's Hospital is the only one to retain smallpox virus in the UK, must be given the means to continue his work (the characterisation of pox viruses by their DNA content). It is not reasonable that he should be hounded into a two-year hiatus in his work because of the laxity of another laboratory. The World Health Organisation, which suffered no strictures in Shooter for its inspection procedures, has designated Dumbell's laboratory as one of the only four in the world that can continue to work on smallpox. He should be able to continue. But perhaps he should be prepared to take up the offer, reported below, of the director of the special pathogens unit at Porton Down, Dr David Simpson, to store his smallpox samples at Porton (whose containment facilities are second to none) and provide him with laboratory space. This would imply that Professor Dumbell's work could continue soon, but in collaboration with Porton staff; and that his animal pox samples—which are not so virulent as *Variola major*—would remain at St Mary's. Such an arrangement might prove an effective compromise, if logic rather than emotion prevails.

Second, the need for improving safety measures and safety education at other laboratories holding category A pathogens (and for that matter the milder category B pathogens too) leads to a number of consequences: that money should be made readily available for increasing laboratory safety (it has not been easily available in the past); that safety officers be provided with sufficient status that they can confront heads of departments on equal terms; and that (we shall argue) a body be set up to provide education and research into biosafety.

That safety money has not come easily in the past is illustrated by the comment, only last week, of Professor Arie Zuckerman of the London School of Hygiene and Tropical Medicine that he could not afford the six white coats per staff member recommended in the Godber report (the result of an inquiry which followed a smallpox outbreak at the school in 1973). Presumably, in the present political climate, money should present no problem. But it must be provided in such a way that there is no conflict of interest over its spending. If it were provided, for example, by the Medical Research Council (MRC), which funds biomedical research, there would be a regular conflict over how much to spend on research and how much on safety. Again, if that money were provided as a block grant to a head of department, there might be an accounting difficulty in determining whether the money had finally been spent on safety or on some other matter. The best solution would seem to be money provided either by the ministry concerned (the Department of Education and Science for universities and the Department of Health and Social Security for hospitals) or better directly by the Health and Safety Executive (HSE) to the safety officer of each laboratory. Then the conflicts of interest in the giving and the spending of money would be minimised.

Status for the safety officer is a thornier problem. It is not merely a matter of equal salaries, but one of prestige, age, and personality. There must be a mutual respect for their individual concerns between the head of department and the safety officer, the one for the academic advancement of the department, the other for the human welfare of its members. Probably the most experienced and knowledgable biosafety officer in this country, Dr Mark Darlow (who was head of the safety section at Porton from 1951 to 1976), believes that the job involves being "a kind of uncle". He is a doctor of medicine, and has something of the manner of a general practitioner—firm yet sympathetic—and believes it vital that a safety officer gets to know the families of the people in his care, and their doctors. He has all the assurance of the medical profession, and one can be quite sure he had the confidence to confront the director of Porton and its scientists when it was necessary. And he was successful, despite the awesome panoply of bugs (from smallpox to plague) that Porton carried. There were very few incidents, and those only due to inevitable human frailty. It is dangerous to argue from one case; but there *are* very few; and it seems reasonable to suggest that a safety officer should always be a doctor. This gives him or her the status and confidence of the profession, and ensures the right motivation; safety is not a business for failed academics.

This leads to the next matter—the need for education and research in biosafety. Darlow's confidence rests not only on his professional sangfroid but also on experiment. In very early days at Porton, for example, he discovered the hardiness of the smallpox virus. He found that it can survive in dust in the corners of rooms, in pockets, even in dust on the top of a fluorescent light. He did experiments with high speed cinephotography on pipetting, and showed that a cloud of tiny droplets floats into the air with every drop released. He had the confidence of facts, and could convince the scientists in his care with evidence. No scientist is going to accept arbitrary controls; there must be adequate reason.

Not only is there now very little research being done (whereas as great deal remains to be learned), but what is known is not widely disseminated. As Dr Darlow wrote prophetically in 1969 "The literature on the subject is in fact very extensive, but many of the publications have appeared in unfamiliar journals and have consequently been overlooked; it is to be regretted that the spilling of ink is so inaudible and that positive action is only stimulated by the resounding spilling of pathogens." (*Methods in Microbiology* vol 1 ch 6; 1969.)

Biosafety is peripheral to the interests of an ambitious academic, and he or she is not going to spend as long reading the safety literature as reading the latest development in his or her field. That much is inevitable. Neither is such a person going to spend much time researching hazards, the reward for which is a human one quite different from the pursuit of academic success. It is therefore necessary to set up a new institution, with its own career structure and motivations, with the objective both of disseminating existing scientific knowledge on biosafety, and, most important for maintaining the liveliness of the body, with the objective of pursuing research into biosafety questions. There are many of these, and not only concerning work with known pathogens; there is a great deal yet to be learned about the safety of work with recombinant DNA, for example.

Further, since one product of such a unit would certainly be ideas for new devices to increase containment and ease research, and since such devices will be in demand, the unit could even generate some of its own income through patents and licensing. If Britain moved fast enough, such a body could become a world leader in the field whose prestige would make it easier to attract the right kind of talents to it: talents in education, medicine, biology, physics, and commerce. The Secretary of State for Education and Science, Mrs Shirley Williams, who recently indicated her willingness to invest large sums of money in research into the hazards of genetic engineering, would be well advised to consider setting up such an institution. It would be an innovation. None yet exists quite like it; neither the National Radiological Protection Board, nor the laboratories of the Health and Safety Executive, nor the research units of the MRC, have such conjoined functions; but the functions must be conjoined as each activity would stimulate the others. Alone, each is a stranded whale.

After continuing research into smallpox; after removing ignorance of biosafety; the third matter we raised was the question of keeping DPAG's successor properly informed. Of course that in itself is not enough: DPAG proved itself unwilling to act. But a dose of 'public interest' and union representation, as on the Genetic Manipulation Advisory Group, should soon cure that. DPAG already had teeth, under the Health and Safety at Work Act; its advice had to be accepted or a laboratory would have to close. Its successor needs no more than that—except information.

DPAG was not well informed. It has emerged now that there is no complete and guaranteed list of the laboratories holding category A pathogens, let alone category B. The Godber working party, which defined the categories, sent out a large number of questionnaires concerning pathogens held to as many laboratories as they could think of. Of 4,016 laboratories questioned, 2,152 did not even reply. Perhaps they held no pathogens; but there is no guarantee of that. Further, DPAG has had only one full time inspector, a retired member of the Public Health Services Laboratory, plus two occasional assistants, to inspect the 115 laboratories holding category A pathogens that *were* unearthed by Godber. At Birmingham, according to Shooter, the DPAG inspectors failed even to consult a checklist of items recommended by the Godber report.

The Health and Safety Executive has a special unit of three microbiologists, but that is less than a year old and is now occupied full time until April inspecting 11 laboratories holding category A human pathogens. (Incidentally DPAG has reduced the list of 12 category A human pathogens defined by Godber to 5, while adding 4 new ones.) This same HSE team also has the duty of inspecting laboratories for the Genetic Manipulation Advisory Group. It is clearly overstretched, and must be expanded by a large factor. But there is another problem: where are people of sufficient quality and knowledge to be found? The HSE function must not be one of rubber-stamping, of accepting the assurances of the 'expert' at the laboratory who rarely turns to the biosafety literature. There may be no more than half a dozen people in the country who really understand the field. These people must be brought together as a matter of urgency, first to assist in training new inspectors for the HSE and second to advise on the setting up of the biosafety research and education unit we recommend. Ultimately that unit can become the source of new inspectors and of biosafety officers.

Finally, there is the question of the need for another public inquiry, as demanded by Mr Clive Jenkins. Our view is that would only confuse a situation where a laboratory is already littered with the recommendations of well-meaning committees. The Godber working party was set up five years ago to clarify the situation and that it did. The failure has been to implement its recommendations properly, which is a failure of government. DPAG should be reconstituted along the lines of GMAG. The HSE microbiological inspectorate should be significantly strengthened. A biosafety research and education unit should be set up. And a way should be found to enable Professor Dumbell to continue his smallpox work rapidly, probably at Porton. These are the ways forward from Birmingham. ☐

On 24 August last year Professor Henry Bedson of the Department of Medical Microbiology at Birmingham University analysed samples from Mrs Janet Parker, a university photographer, and confirmed that she had smallpox. The source of her infection was almost certainly Bedson's smallpox laboratory. Within a few weeks Professor Bedson had committed suicide and Mrs Parker had died. The affair is *sub judice* under the pending court case against Birmingham University under the Health and Safety at Work Act, but despite that Clive Jenkins, leader of the Association of Scientific, Technical and Managerial Staffs last week published his copy of the Shooter report—the confidential government report of the committee of inquiry into the incident. Shooter's indictment is a strong one, and Mr Jenkins believed it in "the over-riding public interest" that he publish it. Here Robin McKie of the *Times Higher Education Supplement*, Mary Lindley, Judy Redfearn and Robert Walgate report.

UK smallpox research could continue at Porton

The unofficial publication of the Shooter report by union leader Clive Jenkins last week is likely to produce swift results in Britain. The recommendation that the nation's only remaining smallpox research unit, at St Mary's Hospital in London, should be moved to an isolated area in the country has already been backed by the local Member of Parliament, Mr Arthur Latham. And the head of the unit, Professor Keith Dumbell, admitted that it would now be impossible for smallpox research to be carried out at St Mary's.

However, later in the week Dr David Simpson, recently appointed director of the special pathogens unit at the Medical Research Establishment, Porton, told *Nature* that he would be willing to receive Professor Dumbell's live smallpox virus at Porton. He could store it there, said Dr Simpson, but he did not want Porton to become "just rent-a-space". Any researcher using Porton in this way would be expected to use and collaborate with Porton staff in research on the material so stored.

Professor Dumbell, on the other hand, believes the scientific case for moving is arguable. "We have in our laboratory all the precautions that we have been able to think up. The chances of a virus escaping are very much smaller than those risks to which we expose ourselves everyday", Professor Dumbell added.

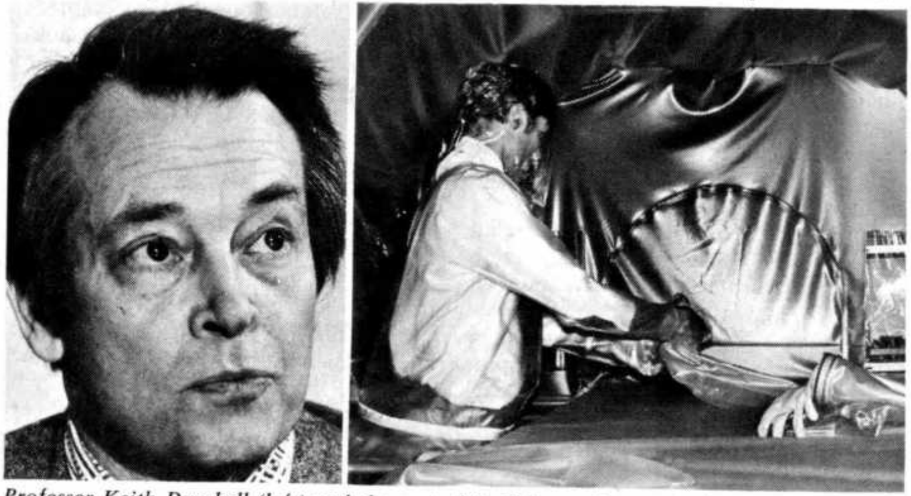
His laboratory was a purpose-built unit designed to cope with smallpox research and took two years to set up. The safeguards—in contrast to the inadequate measures disclosed at Birmingham—include a sealed anteroom; effective laboratory air filtration; antiseptic disposable clothing; a shower room; and regular vaccinations. The move to the country would cost two years' effort that could otherwise be used for smallpox research, he stated.

If the smallpox laboratory at St. Mary's had to close it would be for political reasons, added the school's dean, Dr Harold Edwards. The move to the country would only serve to isolate the smallpox resarchers from colleagues involved in similar areas of work. And Dr Edwards warned that the controversy surrounding the Birmingham smallpox outbreak could lead to growing demands for tighter controls which would diminish the quantity of scientific research in Britain. "The trouble is that increases in controls are not followed by the necessary increases in public funds. Something else has to be taken out of research funds to achieve new safety standards".

Professor Dumbell, who supplied Professor Bedson at Birmingham with 22 virus strains including the one which killed Mrs Parker, admitted there had been a misdemeanour involved when they did not inform the Dangerous Pathogens Advisory Group (DPAG) of the transfer. He thought both of them had assumed the other had notified the group.

Professor Bedson and Professor Dumbell were working on parallel lines of research. At Birmingham, Professor Bedson was using the protein coats of viruses as the basis of an identification process, while Professor Dumbell was attempting a similar technique using the DNA of viruses.

Asked if Professor Bedson could have carried on his work at St. Mary's instead of rushing to complete it at Birmingham, Professor Dumbell said that when the World Health Organisation indicated it was to close down all smallpox research units in Britain, apart from the one at St Mary's, he had been asked by the researchers if he would provide space for them to continue their work. "I answered that I was prepared to do this". □



Professor Keith Dumbell (left) and the sort of facilities at Porton (right) with which he could continue smallpox research.

How the virus escaped

THE Birmingham smallpox laboratory which was the source of the infection which killed Mrs Janet Parker, is a tiny, eight-foot square room in the east wing of Birmingham University's medical school. It is surrounded by a larger room in which experiments on animal pox viruses were performed. Both rooms contain equipment used in normal microbiological research, including safety cabinets with air filters, centrifuges and autoclaves for sterilising gowns and equipment.

The Shooter committee firstly found there was no evidence that Mrs Parker had ever been in the pox virus labora-

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tory suite and they concluded that the virus must therefore have escaped from the smallpox room. The group then discovered that not all smallpox work had been carried out inside the room's safety cabinet and some work with an aspirator to suck off fluid from cell cultures had been undertaken on an open bench.

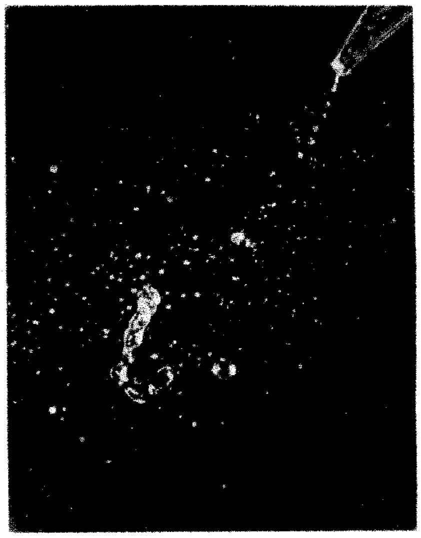
The principal means of transmission of smallpox viruses is probably by aerosol, in tiny droplets of fluid that form when a pipette is used to deliver drops onto a gel or whenever there is splashing of fluid containing the virus. According to Dr Mark Darlow, head of the safety department at the Porton germ warfare establishment for 25 years, the virus is also very stable; so that once airborne it is a potential killer. An efficient air withdrawal and filtration system over any experiment is therefore essential. But many academics still believe that "what was good enough for Pasteur is good enough for me", said Dr Darlow last week.

According to the Shooter report "the opening and closing of the smallpox room door and the passage in and out by whoever was conducting work on the virus would have created the opportunity for any airborne virus to escape into the animal pox room".

The consequent danger was made all the worse because gowns worn in the smallpox room were not removed on leaving.

Even more seriously, the service ducts in the animal pox room and the smallpox room both had gaps which could allow the leakage of viruses. In particular, the telephone room above, which was connected by the duct to the animal pox room, was used frequently by Mrs Parker, when she was telephoning suppliers to order photographic materials. "A check of the orders placed by Mrs Parker during this period reveals that on 25 July she placed an unusually large number of orders. The relevant strain of smallpox virus, Abid, was being handled in the smallpox room on July 24 and 25", the report states.

The committee, although not certain by what route Mrs Parker was infected, concluded that escape via the service duct in the animal pox room was therefore the most probable route of escape of the virus. However, the virus could have reached the corridor outside the laboratory suite and Mrs Parker could have been infected when visiting the inquiry office or the darkroom at the end of the corridor. This was a less likely route, though, and was used by many other persons.



Society for Applied Bacteriology

An aerosol resulting from a bubble burst at a pipette tip when the last drop was expelled. This explains how smallpox virus might have escaped into the air.

And although they could not be certain about the exact route, the Shooter committee was certain that a combination of poor laboratory procedures, amounting to a major breach in containment policy, was responsible for the outbreak. □

All safety nets failed, says Shooter

The report of the Shooter inquiry into the causes of the smallpox outbreak in Birmingham last year finds fault with the three major organisations—the Dangerous Pathogens Advisory Group (DPAG), the World Health Organisation (WHO) and Birmingham University—which were concerned in some way with either running or monitoring the smallpox laboratory at the Department of Medical Microbiology at Birmingham University.

DPAG

The Dangerous Pathogens Advisory Group (DPAG) exists precisely to prevent the sort of event which occurred at Birmingham. It was created in 1975 in the wake of a previous outbreak of smallpox from a laboratory in London in 1973. Shortly after it came into being it began to formulate a code of practice for work with category A pathogens—those recognised as the most dangerous—and to inspect all laboratories known to hold them.

In February 1976, DPAG's inspector visited the Birmingham laboratory. During the time he was there, says the Shooter report, no work on smallpox was being done. He tested the airflow through the safety cabinet and then spent most of his visit talking to Professor Bedson about smallpox work.

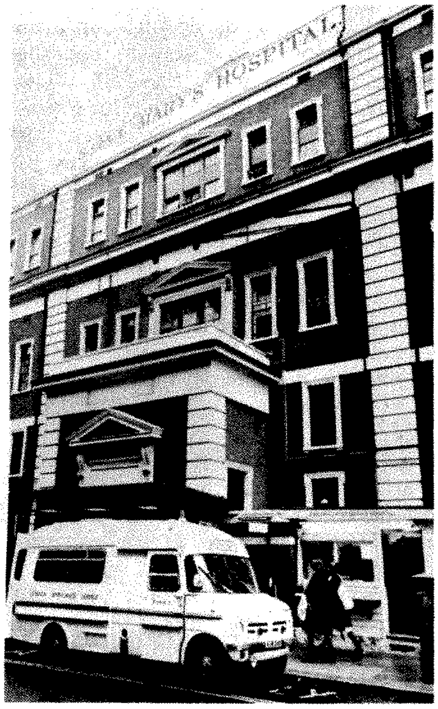
The Shooter report criticises the DPAG inspector for not finding out enough about the "range and extent of the work being done". He did not, says the report, "ask about work with tissue cultures"; neither did he ask about the methods of harvesting virus. "These points seem to us to be of considerable importance", the report goes on, "since one of the unsatisfactory features . . . was the necessity to pass in and out of the smallpox room during the course of work with smallpox to place cultures in the incubators and to use the low speed centrifuge".

Despite the fact that the Birmingham lab lacked some of the facilities then recommended for use with category A pathogens, namely an air lock, shower, changing facilities and double autoclave, the DPAG inspector recommended to DPAG that the laboratory be approved. He based his judgement on Professor Bedson's reputation as an "experienced and safety-conscious virologist" and the fact that the few named people working on smallpox always did so under Bedson's supervision. There was also a "highly efficient vaccination programme" in force.

When DPAG came to discuss the inspector's report (it meets twice yearly) it felt that it could use its discretionary powers—granted to it when it was created—to recommend approval of the laboratory to the DHSS despite the shortcomings. It added, however, that "fresh clearance should be sought in the event of significant changes in staff, facilities or work programme".

The committee of inquiry felt that the inspector's report did not give DPAG sufficient information on which to base a recommendation. It criticises DPAG for not insisting on an inspection report that compared the facilities and procedures with those laid out in its own safety code and recommends that in future DPAG inspectors should compare laboratories' facilities against a detailed check list. It also criticises the way in which DPAG exercised its discretionary powers and recommends that in future "discretion should be exercised by DPAG only if alternative arrangements are in force in a category A laboratory which are able to achieve a degree of safety equivalent to that specified in the safety code."

Since the inspection in 1976, says the report, changes had taken place in the smallpox laboratory. Professor Bedson had ceased to do experiments because of other commitments and most of the work had been taken over by a PhD student. This had not been explained to the DPAG inspector. In addition



St Mary's Hospital, London which houses the UK's only smallpox laboratory.

the volume of work increased. Twenty two additional strains of variola virus were delivered to the laboratory in early 1978.

DPAG, says the report, was notified of none of these changes. The report therefore recommends that as well as notifying DHSS of any changes, category A laboratories should be reviewed annually. It also suggests that notification of intention to hold category A pathogens should be compulsory and that DPAG should start inspecting all other category A laboratories immediately. Finally, it says, the need to work with variola virus in the UK should be reviewed. If it is decided that work should go on, then, the report recommends, the one remaining laboratory in the UK holding the virus, at St. Mary's Hospital, London, should be moved into the country.

Birmingham University

Birmingham University and the Department of Medical Microbiology both had safety policies at the time of the smallpox outbreak which should have had some influence on the way research on smallpox was conducted. The committee of inquiry found, however, that there was no "effective system of determining whether both . . . safety policies were being regularly implemented".

The responsibility of putting the safety codes into practice rested with Professor Bedson as head of department. Responsibility for formulating the safety code specific to microbiological research, however, rested with the committee for the control of pathogenic organisms and infectious materials (CCPOIM), a subcommittee of the university's central safety committee, whose members came solely from the academic staff.

Since the inspection of all departments working with microorganisms by CCPOIM in 1975 there had been no further inspections, says the report. It therefore recommends that "expert safety inspections" should be carried out regularly. It also suggests that a head of department should not be a "member of the team that inspects his own department" (in 1975, Professor Bedson's had been one of the inspecting team), and that the membership of the CCPOIM be extended to include members of the non-academic university staff.

The committee of inquiry also found the experience of the staff working in the smallpox laboratory lacking. Apart from a technician who had worked with smallpox for 11 years and had been trained by Professor Bedson, the other people working on smallpox were a former PhD student, who joined the laboratory in 1974, and a trainee technician who had been working at the lab for about a year. "As far as we know", says the report, the former PhD student "was never formally trained in the special precautions required for work with smallpox viruses". The trainee technician, who joined the lab straight after leaving school, had apparently started working with smallpox virus "only nine months after she had joined the laboratory".

The report says that this state of affairs was inadequate. It recommends that the university review its policy for instructing staff in laboratory techniques and safety precautions and that it ensures that "the staff are not permitted to carry out such work without appropriate . . . supervision".

WHO

The WHO had been corresponding with Professor Bedson about safety without the knowledge of either the Birmingham University authorities or the DHSS.

"It is anomalous", says the report, "that though WHO had decided the work could not be supported after the end of 1978 this was not communicated to DHSS or the university". It recommends that in future "institutions should ensure that all dealings with outside bodies concerning work with safety implications in their departments are monitored by the central administration. . . ." Its recommendation to the WHO is that it should "maintain a closer liaison with the responsible government authority regarding its dealings with category A pathogen laboratories and in particular with regard to the safety of those laboratories". ☐

WHO had serious doubts on safety in smallpox lab

WHEN Mrs Parker contracted smallpox, Professor Bedson and his researchers at Birmingham were working under considerable pressure. They wanted to complete their programme of research on smallpox virus by the end of 1978 when their laboratory was due to close.

The 1978 deadline for the completion of the smallpox work had been decided in 1977 when the World Health Organisation (WHO) informed Bedson that his laboratory had not been chosen as a WHO Collaborating Centre for Poxvirus Research. "It was felt", said the WHO in its letter to Bedson on 16 September, 1977 informing him of its decision, "that . . . the creation of another official centre would give the impression that WHO is itself increasing the danger of laboratory accidents. . . ." In spite of this decision, however, the WHO went on to say that the work at Birmingham was "extremely important and should be supported". It promised Bedson a $7,500 grant for 1977.

In his reply of 4 October, Bedson described the news that his laboratory was not to become a collaborating centre as "a bit of a bombshell". The policy of the UK Department of Health and Social Security (DHSS) was that smallpox research in the UK should only go on in official WHO centres. The implication, therefore, was that Bedson's lab would have to close. This would mean that the work would now have to be done on a shortened timescale, wrote Bedson. "If pressed for a date", he went on, "I would have thought that we should aim to complete our studies with smallpox/whitepox viruses by the end of 1978".

After the WHO's response of 18 October agreeing to Bedson's plan there was a pause in correspondence until late February 1978. From then until 24 August, the day Mrs Parker's illness was diagnosed, all correspondence concerned the visit by WHO inspectors to the Birmingham laboratory on 4 May.

Before the visit took place, Bedson was eager to point out that the Birmingham facilities in no way matched those set out for the WHO's definitive smallpox labs. "It would be expensive and very costly in time", he wrote on 31 March, "if we were to try and establish such a laboratory and quite unjustified in view of our projected halt to the smallpox/whitepox work at the end of the year". Dr I. Arita, chief of the WHO's Smallpox Eradication Unit in Geneva, assured Bedson that the expected benefit of his study "far exceeded the minimal risk" currently present in his laboratory and that the visiting team would understand his situation.

The three inspectors, however, were very critical of the safety precautions taken at the laboratory. They recommended that all mouth pipetting be prohibited, that back fastening gowns which would remain in the laboratory be worn, that hypochlorite solution be used as a permanent barrier in sinks and that gloves be worn for all work with infectious materials.

On 2 June Bedson replied to these criticisms. He accepted the suggestions concerning the use of hypochlorite solution and the wearing of gloves but argued that mouth-pipetting had not been used with smallpox for ten years. The WHO team had observed a 'temporary aberration' which would not happen again whereby mouth-pipetting was used in connection with an animal poxvirus. He also argued that back fastening gowns were used in the smallpox lab and not in the animal pox lab so that they could be distinguished.

One inspector, however, was not satisfied He was concerned about the lack of a shower, the lack of secondary containment in the animal poxvirus lab where smallpox viruses were stored and the performance and maintainence of the biological safety cabinet in the smallpox room. He recommended that the laboratory be upgraded to meet the WHO standards or "discontinue work with variola at the earliest possible date".

The next letter Bedson wrote was on 24 August when he returned from holiday. Later that day he was to find brick-shaped particles characteristic of poxviruses in vesicle fluid specimens from Mrs Parker but at the time he wrote the letter smallpox had not been diagnosed. "There is no question", he wrote, "of our being able to upgrade our facilities to meet the full WHO standards and we are therefore proceeding with our plans to complete our studies with varola/whitepox virus by the end of this year".

What Bedson seems to have never mentioned in any of his correspondence was that the amount of smallpox work in his lab had actually increased substantially. In his letter of 2 June, he even says that there had been "a progressive decline in the scale and diversity of our operations, particularly since 1973". The Shooter inquiry found otherwise. In particular shortly after Bedson wrote this letter the rate of work increased even more as work began on 22 variola strains received from St. Mary's Hospital, London, on 26 May. Dr Mary Darlow, ex-safety officer of the Porton germ warfare establishment, commented last week: "Bedson knew he was backing a lame horse and that it would stumble sooner or later. It stumbled sooner". □

Bedson's concern: could smallpox re-emerge?

RIFT valley fever is a cattle disease with a mortality rate of 80%. It is endemic in Egypt and recently has begun to affect man. The Egyptian authorities estimate that 18,000 people have so far caught the disease but, according to Professor Arie Zuckerman of the London School of Hygiene and Tropical Medicine (LSHTM), a team from the school which recently visited Egypt puts the number at nearer 200,000. Without suitable facilities for diagnosis and research, says Professor Zuckerman, it would be difficult to see how the spread of Rift valley fever could be prevented in Europe once the disease had crossed the Suez canal.

The situation with other diseases is similar. Early diagnosis of rabies or new diseases such as lassa fever or Marburg disease means that they can be contained. Having suitable research facilities means that vaccine and treatment can be developed in case of an outbreak.

Research is also important, however, for predicting new diseases. In the case of smallpox, for example, eradication was possible because of the existence of a very effective vaccine and because it appears to have no animal reservoir or insect vector. What worries microbiologists now, however, is that poxviruses very similar to the smallpox virus which currently affect only animals may begin to affect humans. Research on the similarities between different poxviruses and their relationship to smallpox might therefore indicate how likely this is.

The first poxvirus to be identified in a non-human primate was monkeypox virus which was isolated in 1958. Since then it has been isolated from non-human primates on several occasions and has been responsible for about 30 cases of human monkeypox in West and Central Africa between 1970 and 1978. Fortunately the spread of human monkeypox from man to man is extremely rare.

Another poxvirus very closely related to smallpox is whitepox. It was first isolated from the kidneys of cynomolgus monkeys in 1964 and it is still impossible to distinguish it from smallpox virus by laboratory tests.

The work at Birmingham centred on the identification and differentiation of poxviruses. Early work had tried to distinguish viruses by looking at the enzymes they produce. More recent work, however, had used a technique called polyacrylamide gel electrophoresis (PAGE) to separate out the polypeptide constituents of viruses and then compare them.

By mid-1978 the Birmingham team had completed comparisons of 41 different poxviruses using PAGE techniques. It had found that all but three could be put into one of four basic categories. The smallpox/whitepox category of viruses could be subdivided into types A and B. When a report of the findings was written for the WHO much more work was planned on the differentiation of types A and B smallpox viruses. Most of the B viruses seemed to be of Middle Eastern or Pakistani origin whereas the A viruses had mainly originated in Africa. Whitepox viruses were also found to subdivide into types A and B, the B type originating in Asia and the A type in Africa. "This supports the view", said the report "that these (whitepox viruses) must be regarded as smallpox viruses".

After the extensive study of smallpox/whitepox viruses which was to have finished at the end of 1978, the Birmingham laboratory was planning to continue its study of animal pox viruses Minor differences had been observed between some of the cowpox-like viruses and some of the monkeypox-like viruses, said the report, and those should be the subject of further study.

- The WHO says that to the best of its knowledge the only laboratories holding smallpox viruses are the following National Institute for Virology, South Africa; Centre for Disease Control, Atlanta, USA; American Type Culture Collection, New York, USA; Walter Reed Army Institute of Research, Washington, USA; Bayerische Landesimpfanstalt, Munich, West Germany; Institut fur Schiffs und Tropenkrankheiten, Hamburg, West Germany; Rijks Institut voor de Volksgezondheid, Bilthoven, Netherlands; Laboratory of Smallpox Prophylaxis, Research Institute of Viral Preparations, Moscow, USSR; Virology Department, St Mary's Hospital Medical School, London, UK; Institute for Control of Drugs and Biological Products, Peking, China. □

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Health in the laboratory

FOR more than 20 years both government and unions have been concerned about standards of safety in British laboratories and the health of people working in them. The first major stimulus came in 1957 when D. D. Reid, of the London School of Hygiene and Tropical Medicine (LSHTM), reported that the incidence of tuberculosis among medical laboratory workers was higher than expected. As a result, a working party of the Public Health Laboratory Service (PHLS) made recommendations for safe working in laboratories handling tuberculous material. These were revised in 1968, but adverse reports continued to appear sporadically, and in 1970 the Department of Health and Social Security (DHSS) commissioned a full investigation from the Trades Union Congress (TUC) Centennial Institute of Occupational Health at LSHTM. This was carried out between 1971 and 1974 and the full report results appeared as a thesis in 1975. As far as the author is aware, the DHSS has done no more than file the report.

The investigation included retrospective surveys of the incidence of four infectious diseases and the standards of health care and safety in the laboratories of the National Health Service (NHS, consisting of hospital laboratories), the National Blood Transfusion Service and the PHLS (Harrington, J. M., & Shannon, H. S. *Brit. Med. J.* **1**, 759; 1976). The surveys involved 24,000 workers, constituting 85% of the total in those laboratories. The results for the infectious diseases are summarised in Table 1.

The totals for tuberculosis in England and Wales and in Scotland represented, respectively, a 5.4 and a 4 2 times greater risk of acquiring the disease among laboratory workers than the general population. It was not possible to make a similar assessment for the other infections, but the conclusion was that hepatitis possibly remained an occupational hazard for laboratory workers, while shigellosis may have been more prevalent among them than among the general population. All four diseases seemed most likely to afflict technicians

Variable standards of safety and health care were reported (Harrington, J. M. & Shannon, H. S. *Brit. med. J.* **1**, 626; 1977). Less than half of the laboratory workers had been examined medically before employment and a similarly small proportion had been offered vaccination. The length of service of the workers indicated that the standards had not improved during the previous 10 years and had probably deteriorated Scottish laboratories proved to have a slightly better record in safety control than their English and Welsh counterparts, and PHLS laboratories did better than those of the NHS, providing more formal safety training.

Mouth pipetting was still being practised in 65% of English and Welsh laboratories and 28% of Scottish laboratories. Although protective clothing was usually available, its use was compulsory in only one third of English and Welsh laboratories and two-thirds of Scottish laboratories, even when procedures involved were known to be hazardous. The servicing of safety cabinets was inadequate in two-thirds of Scottish laboratories and 40% of English and Welsh laboratories, and known carcinogens were still being used in a small proportion of laboratories.

Another phase of the investigation involved a prospective survey of accidents and absence from work due to illness in 10% of the laboratories surveyed previously (Harrington, J. M. MD thesis Univ. London, 1975). Between April 1973 and March 1974 records were kept for 2,520 workers in 39 laboratories. Absence was generally lower than in other British industries. The only possible sign of a link with occupational hazards was provided by infectious and parasitic diseases, which constituted the chief cause of absence.

During this phase of the investigation 627 accidents caused injury in the 39 laboratories and one in four of the workers was affected. Technicians—64% of the population—suffered 76% of the injuries, including all nine of those due to mouth pipetting. The most common injuries among all groups of laboratory workers were lacerations, splashes and burns to the hands or eyes, most of them relatively trivial.

The remaining phase of the investigation was a retrospective study of mortality among 156 pathologists (dying between 1955 and 1973) and 154 medical laboratory technicians (dying between 1963 and 1973) (Harrington, J. M. & Shannon, H. S. *Brit. med. J.* **4**, 329; 1975). Both populations experienced lower mortality than the general population, as would be expected because any occupational group is likely to be healthier than the whole population.

The only examples of higher incidence among the laboratory populations were for lymphoma and leukaemia, which killed eight male English pathologists compared with the expected 3.3, and suicide which in England and Wales was the cause of death in nine male pathologists. compared with the expected 3.4, and nine male and six female technicians, compared with the expected 4.7 and 1.5, respectively. Suicide proved to be the commonest cause of death among English female technicians. Their easy access to poisonous chemicals was thought to be the most likely explanation. ☐

Table 1 Infectious diseases in laboratory workers in England and Wales in 1971 and Scotland in 1973.

Infection	Medical staff	Scientific staff	Technical staff	Other	Total
Tuberculosis	1 (1)	1	14 (2)	2	18 (3)
Hepatitis	2 (1)	1	27 (2)	5	35 (3)
Shigellosis	1	3 (1)	29 (7)	4	37 (8)
Brucellosis	–	–	1	–	1

Figures for Scotland are in parentheses.

Category A laboratories

Laboratories approved by the Dangerous Pathogens Advisory Group to hold specific Category A pathogens, (in parentheses) as at December 12, 1978.

Department of Microbiology
University of Reading (Rabies)

Central Public Health Laboratory
Colindale (Rabies)

Central Veterinary Laboratory
Weybridge, Surrey (Rabies)

Evans Biologists Ltd
Liverpool (Rabies)

Animal Virus Research Institute
Pirbright (Rabies)

Lister Institute
Elstree (Rabies)

Institute of Virology
University of Glasgow (Rabies)

Microbiological Research Establishment
Porton, Wilts. (Rabies, Lassa fever,
Marburg, Simian herpes B, Crimean
haemorrhagic fever (Congo),
Venezuelan equine encephalitis, Ebola)

London School of Hygiene and
Tropical Medicine
Winches Farm, St Albans (Crimean
haemorrhagic fever (Congo), Venezuelan
equine encephalitis)

Department of Virology
St Mary's Hospital Medical School
London (Smallpox)

Wellcome Research Laboratories Ltd
Beckenham, Kent (Rabies)

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American Association for the Advancement of Science meeting in Houston, Texas.

Reports by David Dickson

AAAS president calls for closer university/industry links

WITH US industry under pressure to remain competitive, and universities facing problems in securing research support from conventional sources, the time has come for a closer relationship between the two, according to Dr Edward David, president of the American Association for the Advancement of Science.

Close links between the industrial and academic communities could help to preserve important features of the research system: a pluralism of funding sources, leading to greater local autonomy in the conduct of research and development programmes; a more competitive environment, based on technical excellence; and a dependence on "high achievers" able to provide scientific leadership.

If basic research was defined not as research carried out with no particular goal in mind, but rather as any research leading to a greater understanding of natural and social processes, regardless of the motive, then the amount of such research being carried out by industry was larger than current definitions indicated. Industry had also widened its research interests beyond just improving the technology of production lines. "Today there is a much broader point of view, ranging from the sociology of customers, to research on the R&D process itself." Industrial research was increasingly using the basic research paradigm in forwarding its function, and it would not be incompatible to couple this directly with academic interests.

In the 1950s and 1960s, industry support for university research had been primarily philanthropic in nature. Now a number of companies were exploring ways of setting up long-term cooperative arrangements with universities. "Ten years from now I expect to see a vigorous community of industrial/academic scientists, with academics providing the ideas and industry providing the vehicle for their commercialisation. This will certainly be an improvement on the situation that we have now. And it will be more in keeping with the situation facing both sides in the 1980s." ☐

US to brief scientists on human rights

THE US State Department is preparing briefing materials for scientists and others who participate in official exchanges, describing the goals and objectives of the US human rights policy, and responding to questions about the legality and safety of activities in defence of colleagues abroad.

Mrs Roberta Cohen, of the Department's Bureau of Human Rights and Humanitarian Affairs, told a session on international freedom and responsibility that US Government agencies and representatives had been instructed not to discourage or prohibit exchange participants from contacting private Soviet citizens. "We in the Bureau of Human Rights take it as an obligation of the Government to correct any wrong impression created that it is seeking to discourage scientists on exchange programmes from efforts to defend foreign colleagues," she said.

The Government asked only that contacts of potential sensitivity, particularly in tense periods, be discussed in advance with Administration officials, "in order to ensure that American scientists involved have full information assessing the potential risks".

A blanket halt to exchanges had distinct disadvantages, ranging from a reduction in the exchange of ideas to raising the spectre of Government interference. Boycotts of meetings were not likely to be as productive as participating and expressing human rights concerns, she said. ☐

Rethink for research funding

A LARGE proportion of federal funds for university research should be distributed to institutions on a "formula" basis calculated on proven capability and productivity. Such a "deregulated and decentralised" system for research grants would avoid much of the unnecessary work involved in the current grant-proposal/peer-review process, Professor Rustum Roy, chairman of the science, technology and society programme at Pennsylvania State University, told a session on federal research and development funding. He also suggested a three-year national commission to set national priorities in research and development, with a strong input from the scientific and engineering community.

Professor Roy said existing research and development mechanisms were designed for the "golden age" of science in the 1950s and early 1960s, but were no longer appropriate. The new social setting for R and D included: public recognition of the "ambivalence" of science and technology, rather than seeing either as solely beneficial; widening gap between science and the public; a recognition that there is no simple relationship between support for basic science and a healthy economy; and the fact that the system had not led to maximum innovation in the industrial sector.

The problem lay not so much in getting more money for basic science—"possibly the least important question"—as in designing a more efficient system for the disbursal of funds, where radical changes could produce a major increase in productivity.

A clearly stated rationale for the support of science was also needed. Most important would be contributions to nationally-defined objectives such as defence, health and energy; then would follow science for graduate education, science for state, community and local needs, and finally "science for science's sake". Congressman George E. Brown, a leading member of the House of Representatives Committee on Science and Technology, pleaded for a more sophisticated strategic planning operation for the national scientific effort.

"It is impossible to develop a new rationale for the support of science and better mechanisms for carrying this out without a strong and well-defined central planning authority in the scientific sphere," he said. Some members of Congress had hoped that this role would be played by the President's Office of Science and Technology Policy when it was reestablished in 1976. However, many of the policy and planning tasks envisioned for OSTP had gone to other offices further from the executive centre.

Another need was to develop mutually reinforcing networks of research institutions. "The capabilities of various federal, state and local laboratories must be better linked, both in terms of facilities and people. Only when we have taken such moves can we persuade the public and Congress that critical gaps in research remain and are in need of support." ☐

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Assessment of cancer risks comes under attack

EXTRAPOLATING downwards from the effects of exposure to high levels of ionising radiation is an inadequate basis for predicting the dose-response relationship for the incidence of cancer at low levels of exposure, according to Dr Alice Stewart, of the Department of Social Medicine at Birmingham University, England. Such extrapolation would only be valid if cancer was the sole effect of radiation. However there were other indirect effects which would be hidden by the high-dose data, but which would make a linear dose-response relationship untenable, Dr Stewart told a session on epidemiology studies of low-level radiation exposure.

Recent analysis of medical data collected on workers at the US Government's nuclear facilities at Hanford in Washington State, and therefore based solely on low levels of exposure, indicated that a curvilinear model for the dose-response relationship was better than a linear model. The best fit was given by a curve in which the response was proportional to the square root of the dose.

Dr Stewart said medical considerations raised questions about using data based on survivors of the atomic bomb explosions at Hiroshima and Nagasaki as a basis for predicting the effects of low-level exposure to ionising radiation. "Claims that the mortality of A-bomb survivors between 1950 and 1974 from diseases other than cancer is unrelated to ionising radiation gave a false impression. This was due partly to the fact that direct and indirect effects of radiation delivered at high dose-rates had similar consequences, and partly to the ease with which other diseases could prevent cancer induction by radiation—or other carcinogens—from developing into clinical cancer".

Prevention at this stage was usually the result of a non-cancer death coinciding with a period of cancer latency. "Optimal conditions for observing cancer effects of radiation are therefore those which combined low rates of general mortality with small doses of radiation delivered at slow dose rates."

Dr Stewart said that these were the conditions surrounding data collected by Dr Thomas Mancuso, of the University of Pittsburgh, on the health of workers at the Hanford plant, which have recently caused considerable controversy by raising serious doubts about the adequacy of current protection measures

Mr George Kneale, a Birmingham University statistician, who has been helping Dr Stewart to analyse Dr Mancuso's results, told the session that the most recent analysis of the data provided a doubling-dose for the incidence of radiation-linked cancers of between 2 and 150 rads, whereas calculations based on a linear extrapolation from data on A-bomb survivors indicated a doubling-dose of between 150 and 200 rads. "On internal evidence the Hanford survey rejects the possibility that the true risk may be as low as it seems from the A-bomb survivors, but we cannot say precisely what the true risk is, apart from the fact that it appears to be higher at low doses than extrapolation downwards from high doses would indicate."

Doubts about the validity of conclusions of epidemiological surveys based on relatively small sample data were raised by Dr Charles E. Land, of the National Cancer Institute's environmental epidemiology branch. Dr Land warned that an inadequate sample size severely reduced the chances of correctly rejecting a false hypothesis of no dose effect at low levels of exposure. "Failure to reject is not strong evidence for the null hypothesis, so the study results are likely to be inconclusive—a wasted effort", he said.

"We do not have the resources for adequate epidemiological studies of populations exposed to low levels of radiation, and if we should try to do them anyway we would run considerable risk of obtaining misleading results, results that would derive at least some credibility from the vast effort of obtaining them," he said. ☐

Meanwhile, back in Washington . . .

DNA critics appointed to advisory committee

Two active participants in recent debates over the adequacy of guidelines for research using recombinant DNA techniques have been appointed to the committee responsible for advising the director of the National Institutes of Health on a number of important aspects of such research

The two—Dr Richard Goldstein, assistant professor of microbiology and molecular genetics at Harvard Medical School, and Dr Sheldon Krimsky, acting director of the program in urban, social and environmental policy at Tufts University—are among 14 new members appointed to the Department of Health, Education and Welfare's Recombinant DNA Advisory Committee by HEW Secretary Joseph A. Califano.

The appointments follow Mr Califano's announcement last month of a number of changes to existing arrangements for regulating recombinant DNA research. The advisory committee is responsible for giving advice on new types of bacteria for use in such research, on whether certain presently prohibited experiments should be conducted, whether additional categories of research should be exempted from the guidelines, and on possible future changes in the guidelines.

Mr Califano also announced that the size of the advisory committee was to be increased to 25, and that the scope of its membership was to be extended to give greater weight to non-scientific representation. The new members of the committee include a professor of education, a professor of law, a prominent environmentalist and a laboratory technician.

In addition to Dr Goldstein and Dr Krimsky, the new members include: Dr Karim Ahmed, senior staff scientist with the Natural Resources Defense Council; Zelma Cason, chief of cytotechnology in the department of cytology at the University of Mississippi Medical Center; Patricia King, professor of law at Georgetown University Law Center in Washington; Dr Samuel Proctor, professor of education at Rutgers University; and Ray Thornton, retiring member of the US House of Representatives and chairman of the House subcommittee on science, research and technology in the last Congress.

The new scientific and medical members are: Dr David Baltimore, (MIT); Dr Francis Broadbent (University of California, Davis); Dr Richard Novick (New York Public Health Research Institute); Dr David Parkinson, (University of Pittsburgh); Dr Damon Pinon, (University of California); Dr Luther Williams (Purdue University); and Dr Frank Young (University of Rochester).

There is thought to have been considerable controversy over some of the other names of "public interest" representatives suggested for possible membership of the committee, particularly since some scientists involved in the research felt that too large a non-technical component might impede the committee's functioning. ☐

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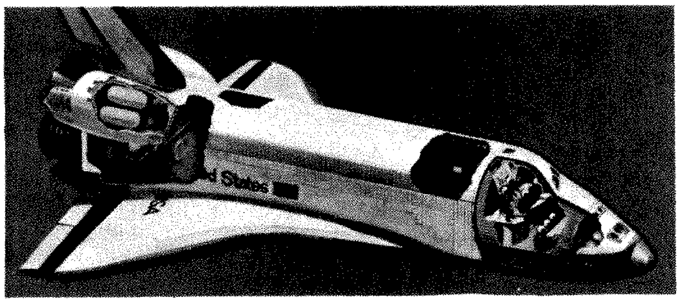
news in brief

Engine failure threatens further delay to space shuttle: An engine failure during testing is threatening further delays to the maiden flight of the US National Aeronautics and Space Administration's space shuttle (artist's impression *below*), currently scheduled for 28 September. The failure occurred during tests two weeks ago near St. Louis, Missouri, when a fire in the high pressure turbo-pump that feeds oxygen into the combustion chamber caused the engine to explode.

Space shuttle's initial flight has already been postponed from March following other problems in the engine development programme, and the delays have added extra costs to the programme. According to NASA, the test engine was heavily damaged in the explosion, and it is expected that it will take three to four weeks to repair the test stand. It is not yet known whether the engine failure will affect the current launching schedule. The agency issued a statement last week saying that an assessment of the impact to the engine test programme and to the first flight schedule was now being made.



Argon isotope ratio on Venus differs from Earth: Data from the latest Soviet Venus probes, Venera-11 and -12, which reached the planet at the end of December "astounded everyone"—according to a report from the flight control centre, broadcast a few hours after the second descent module had landed. The surprising result, it appears, was the unexpectedly high ratio of ^{36}A to ^{40}A in the atmosphere—some 200–300 times higher than the ratio of argon isotopes in the terrestrial atmosphere.

Perhaps the most surprising thing about the result, however, is that this value is a close confirmation of the result obtained by the latest US probe which reached Venus some three weeks before the Soviet modules. If the flight control centre was indeed "astounded", this surely reflects on the rate at which the Soviet team get access to the data from US missions—a point which could well be taken up in any future discussions of peaceful cooperation in space research.

According to the US researchers, the new data suggests a two-stage accretion process of planet forming. Professor Zolotukhin, Deputy Director of the Space Research Institute of the Soviet Academy of Sciences, seems to confirm this view, saying that the Venus argon ratio is "important for the study of the evolution of the planet".

A more popular report from the flight control centre, however, took a somewhat different line. According to commentator Nikolai Zheleznov, the argon value, taken in conjunction with the high surface temperature and dense atmosphere, suggests that the process of chemical evolution of the substances comprising the planet was "lengthened", thereby making the "paths of development" of Earth and Venus "diverge".

UK scientist wins redundancy pay: A cell biologist has won £400 redundancy pay from his former employer, the Imperial Cancer Research Fund. The ICRF has a clause in its contracts of employment for untenured staff which states that they waive their rights to redundancy payment. Redundancy claims therefore are complicated and must be based on technicalities in the law. In this case the contract was for one year. The waiver clause is invalid for contracts of less than two years. The ICRF could have argued that the scientist's employment, consisting of one two-year contract and two one-year contracts, constituted more than two years total employment but decided not to contest the claim. Workers under age 41 are entitled to one week's pay for each year of employment not exceeding £100 per week. Of the ICRF's 128 scientific staff 40% are tenured, 15% on limited-term contracts are eligible for tenure and 45% are employed on non-renewable fellowships.

Sun powered telephones in Jordan: Over 80 solar powered telephones have been installed along Jordan's roads to provide motorists with an emergency telephone service. At the top of each telephone pole is a plate of solar cells which convert sunlight into an electric current that charges a storage battery of 36-hour capacity. The telephone is connected to a UHF transmitter powerful enough to reach the nearest microwave installation. Each telephone is equipped with a series of push buttons marked for the type of emergency service needed. At the push of a button the corresponding signal is relayed to an operator at one of three central exchanges. The system is especially designed for foreign speakers but a receiver is available for voice communication.

Saudi Arabia creates a new science and technology centre: The Kingdom of Saudi Arabia has announced the formation of an independent Saudi National Centre of Science and Technology. The centre will be responsible for support of scientific research and for coordination of existing scientific agencies in accordance with Saudi Arabian Development Plans. The brief of the new centre includes starting special research laboratories appropriate to the research needs of the kingdom, assistance to the private sector for research into increasing industrial and agricultural production, and support for specific research projects including grants to develop local skills for scientific research.

The centre is to seek joint projects with international scientific agencies and to propose a detailed plan "to realise the kingdom's objectives in technological fields". The local scientific community has welcomed the centre as an important part of Saudi Arabian social and economic development.

EEC announces fusion programme for 1979–1983: The total cost of the 1979–83 fusion programme proposed by the European Commission is 921 million European units of account (1 EUA=$1.3). Of this sum 185 million EUA is scheduled for the Joint European Torus (JET) of which 80% will be paid by the commission. The remaining 736 million EUA will be spent over the next five years on the rest of the EEC's fusion programme which includes research on Tokamaks, new plasma heating systems, inertial confinement, tritium technology and materials research.

Already 70% of the contracts have been placed for the fabrication of the machine parts of JET. In February the first stone will be laid for its building in Culham near Oxford. Construction of the machine itself will begin in 1981 and is expected to be finished by 1983 when the first tests can begin. JET is to be built with the participation of all nine community countries plus Sweden and (shortly) Switzerland. After a three-year struggle, the Council of Ministers decided in May 1978 to site the machine in the UK at Culham.

news and views

Amorphous silicon: where now?

from S. R. Elliott

ONE of the more extensively studied materials in solid-state research at present, certainly in the field of amorphous semiconductors, is the amorphous silicon–hydrogen alloy system. Upward of 30 laboratories around the world are now working on these materials. The reasons for all this activity are, of course, not hard to discern; they are principally its photovoltaic properties and their possible exploitation in solar energy conversion (see for example *News and Views* **275**, 93; 1978). The attraction lies chiefly in the fact that large area, thin film solar cells could be made relatively cheaply (particularly compared with conventional single crystal devices). However, at present amorphous devices can manage at best only $\simeq 6\%$ conversion efficiency (in contrast to crystalline Si devices that reach $\simeq 16\%$), and it seems that such limitations could be inherent to the process which is currently used to produce these amorphous silicon (a-Si) films.

Spear and his co-workers at the University of Dundee announced in 1975 that by producing a-Si films by means of the decomposition of silane (SiH_4) using an r.f. glow-discharge, doping could easily be achieved by admitting phosphine (PH_3) or diborane (B_2H_6) to the gas flow producing samples which were n-type (the majority current carriers being electrons) or p-type (the majority carriers being holes), respectively. This offered many prospective applications using for example p-n junctions, and was a very significant advance over previous methods of producing a-Si (by vacuum evaporation or sputtering in argon), in which doping was not achieved upon incorporation of the appropriate impurities. The broken (or dangling) bonds introduced in large densities ($\simeq 10^{19}$ cm^{-3}) by these latter preparative techniques give rise to electron states lying deep within the forbidden energy gap between valence and conduction bands, which can trap any excess carriers introduced by dopant atoms. Indeed, these defect states control to a very large extent much of the electronic behaviour of such films.

Studies by Fritzsche (University of Chicago) and Brodsky (at IBM) and others have indicated that a relatively high concentration of hydrogen ($\simeq$ 5–10 at.%, sometimes more) remains within the films prepared by glow-discharge decomposition, a finding not altogether surprising in view of the fact that the decomposition of silane is thermodynamically endothermic. It therefore appears that the presence of hydrogen is in some sense essential to the favourable properties of glow-discharge a-Si. Naively, this can be explained by the notion of hydrogen bonding to the dangling Si bonds, thereby removing their electron states from the energy gap, and thus making doping possible. However, this requires at most $\simeq 0.1$ at.% hydrogen, far less than the amount that is found experimentally. It must be assumed that the balance of the hydrogen is bonded into the random network during growth. The importance of hydrogen in producing films that have favourable photovoltaic properties is underlined by the work of Paul and coworkers at Harvard University, who have produced a-Si:H alloys by sputtering in an atmosphere comprising both argon and hydrogen, which can be doped, and which therefore behave in a manner somewhat similar to those produced by the glow-discharge decomposition of silane.

Despite the promising progress made so far in the field of photovoltaic applications, several problems remain with glow-discharge-produced a-Si. Numbered amongst these are: (1) the variation in properties with the conditions of deposition (for example, the hydrogen content, and hence allied properties, is a sensitive function of substrate temperature, gas pressure, r.f. power); (2) photo-induced changes, such as the metastable decrease in conductivity after optical illumination (Staebler & Wronski *Appl. Phys. Lett.* **31**, 292; 1977); (3) the relative inefficiency of doping, in that only $\simeq 30\%$ of impurity atoms act as donors or acceptors (that is $\simeq 70\%$ bond according to their normal valence and are not forced into tetrahedral environments on substituting for Si atoms) and the Fermi level can only be moved to within $\simeq 0.2$ eV of the conduction or valence band edge; and (4) short minority-carrier lifetimes. In this context, a recent publication by the Harvard group (Paesler *et al. Phys. Rev. Lett.* **41**, 1942; 1978) entitled 'New development in the study of a-Si:H alloys: the story of O', is of interest. This group concentrates on material prepared by sputtering in partial hydrogen atmospheres. They point out that many of the differences in behaviour exhibited by these alloys, compared with those prepared by the glow-discharge decomposition of silane, could be ascribed to the presence of oxygen in the latter; the poorer vacuum and often less pure gases used in the glow-discharge process immediately imply this. To test this hypothesis, samples were produced by sputtering in an argon atmosphere containing both hydrogen and oxygen; the resulting films contained $\simeq 1$ at.% oxygen. They found that the oxygenated films exhibited a much larger photoconductivity and photoluminescence and were relatively insensitive to the partial pressure of hydrogen used, in marked contrast to oxygen-free films; in short, the oxygenated sputtered films more closely resembled glow-discharge samples. It was concluded that in some way the presence of oxygen (along with hydrogen) removes non-radiative recombination channels, perhaps by saturating any dangling bonds present.

Evidence was also presented for the importance of structural defects in the photoluminescence process. This is a much debated subject at present; controversy centres on whether electron states in the energy gap of a-Si:H alloys are introduced by hydrogen atoms attached to Si atoms other than

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by simple covalent bonds (Appelbaum *et al. Phys. Rev. Lett.* **39,** 1487; 1977; Singh *et al.Phys. Stat. Sol.* (b) **81,** 637; 1977; Fisch & Licciardello *Phys. Rev. Lett.* **41,** 889; 1978), or whether simple reconstructed Si dangling bonds containing either one or two electrons (and hence positively or negatively charged respectively), give rise to such states, as proposed by the author and Adler (to appear in *Philos. Mag.* and *Phys. Rev. Lett.,* respectively). The short minority carrier lifetime, one contribution to poor device performance, can for example be attributed to such states within the gap, of whatever origin. The Harvard group's paper indicates a possible solution to the problem of the nature of the defects, although it does not resolve the issue.

However, all such considerations may be made redundant with the announcement by Ovshinsky (*Nature* **276,** 482; 1978) that a-Si alloys produced from a mixture of silicon tetrafluoride (SiF_4) and hydrogen, and therefore containing both fluorine and hydrogen, are markedly superior to those produced by the glow-discharge decomposition of silane. Doping efficiencies are larger, photostructural changes are absent, and deposition conditions only weakly affect the properties of these a-Si-F-H alloys, in sharp contrast to the problems that bedevil silane glow-discharge films. Perhaps here lies the road ahead for amorphous silicon alloys. ☐

Ecological interactions in the Southern Ocean

from Robert M. May

THE Southern Ocean sustains a great variety of vertebrate species, including whales, seals, birds and fish.

Very many of these species share a common resource, the shrimp-like krill, as the main component of their diet. The mechanisms whereby these competing species coexist are of interest in illuminating general ecological principles. Viewed in this light, the massive depletion of the populations of large whales constitutes a vast, and sadly ill-documented, ecological experiment.

Whales

As a group, the whales probably originated about 60 million years ago. Their patterns of distribution in the Southern Ocean must have been subject to many changes in the past few hundred thousand years, with the comings and goings of the Ice Ages. The large baleen whales in this area comprise six species and one subspecies—the southern right, humpback, fin, sei, minke, blue, and the subspecies pigmy blue. There is one large toothed whale, the sperm; only adult males enter Antarctic waters. In addition there are nine species of small toothed whales, which include the killer whale *Orcinus orca,* the southern white-sided dolphin, and two species of beaked whales. Little is known about the ecology of these smaller toothed whales.

Laws (*Phil. Trans. Roy. Soc. Lond.* **B279,** 81; 1977 and in *Adaptations Within Antarctic Ecosystems* (ed. Llano), Smithsonian Institution, 1977, hereafter referred to as Laws I and II, respectively) has drawn together much earlier work, to give a fascinating account of the mechanisms of ecological separation among these whale and other species.

The standing crop of zooplankton, mainly krill, in the Antarctic is at least four times that in the tropics, but it is a highly seasonal resource for whales. In addition to seasonal changes in krill abundance itself, the krill zone is nearly all covered by pack ice in the southern winter; in summer the ice retreats and 80–90% of the krill zone is exposed when the pack ice is at its minimum in March. Most of the baleen whales make long migrations, feeding in summer in the food-rich Antarctic waters, and breeding in winter at low latitudes, where they of necessity fast and rely on energy stored as fat (blubber). A conservative estimate of the weight lost in winter is 20%, which compares with many terrestrial mammals, such as deer. The migrations appear to be staggered, with the different species arriving in the Antarctic at different times in the summer. The blue appears earliest, the fin and humpback next, and then the sei. These waves of migration also reflect the extent to which the different species penetrate into colder waters; the blue appears first and follows the retreating pack ice south, while the other species fill in behind it. Ohsumi *et al.* (*Sci. Rep. Whales Res. Inst.* **22,** 75; 1970) estimate that the highest densities of sei whales are found in the circumpolar zone spanning 40-50°S, fin in 50-60°S, and blue and minke in 60-70°S. The pigmy blue whale seems confined to waters north of 54°S. The southern right whale is probably not migratory and may feed all year in the zone 30-50°S (see Fig. 1).

With the conspicuous exception of the minke, the timing and extent of the migration patterns correlate with the body sizes of the species, and with their percentage proportions of meat and blubber: blue, 39% meat and 27% blubber; fin, 46% and 24%; sei, 59% and 17%; minke, 62% meat and 15% blubber. Although the minke penetrates far south, its low proportion of blubber and small size suggest it is a more continuous feeder than the larger and more migratory species. It also has a higher population turnover, with a 1-year reproductive cycle in contrast to the other whales' 2-year cycle. Within this set of species, we may view the minke as the r-selected opportunist, superimposed on the smooth pattern of spatial and temporal segregation manifested by the other baleen whales.

The baleen whales are the top pre-

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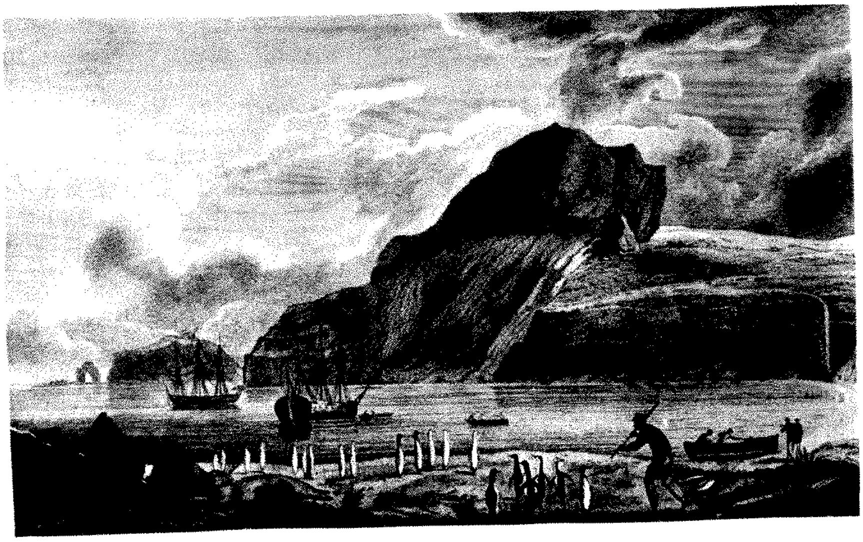
A view of Christmas Harbour in Kerguelen Land. From a contemporary account of Cook's third voyage.



dators in a very short food chain, filter-feeding on planktonic crustacea. The rough geographical zones occupied by krill, the major species of these crustacea, are also indicated in Fig. 1. The baleen plates of the various whales are adapted to the size of the food taken: the right whale, which feeds primarily on small copepods, has very fine long baleen and the mouth is enlarged to accommodate them; the filtration by the plates of the sei, minke, humpback, fin and blue is increasingly coarse. Feeding behaviour also varies. The fast-swimming blue, fin, minke and humpback whales swallow in discrete mouthfuls which is more effective with swarming food organisms such as krill, while skimming with the mouth closing at longer intervals (as done by right whales) works better against dispersed organisms such as copepods. The sei uses a combination of the two.

The main sperm whale stocks are found outside Antarctic waters, and females rarely penetrate latitudes beyond 45°S. Solitary males begin to show migratory behaviour as they attain sexual maturity (starting around age 12 years), and their propensity to migrate to Antarctic waters in the summer increases as they get older, levelling off around age 25 years when they may attain the status of harem master in the breeding herds at low latitudes. Sperm whales feed largely on squid species found in temperate and Antarctic waters.

Although not much is known about them, the smaller toothed whales are probably resident in Antarctic seas all the year round.

Rough estimates of the past and present numbers and biomass of large whales in the Antarctic are given in Tables 1 and 2, along with estimates of their food consumption (simplified from Laws I). These numbers are not graven in stone; the many uncertainties in the data are such that no two published tables are identical. A list of the main assumptions and uncertainties is as follows. One-half the total sei whale stock, and one-third of the 'exploitable male' sperm whales are assumed to feed in the Antarctic, south of the Antarctic Convergence. The figure of 10,000 blue whales is higher than the 1978 IWC figures, as is their mean weight of 83 tonnes; Masaki and Yamamura (*Rep. int. Whal. Commn* **28**, 258; 1978) give 4,500 at an average weight of 62 tonnes. The minke figures, both past and present, are very uncertain, and estimates range widely (the minke have only recently been exploited, and most information derives from commercial whaling). With the exception of the minke, which is assumed to feed all year in Antarctic seas, the whales are taken to spend 120 days feeding in the Antarctic.

Table 1 Initial whale stocks

Species	Population (thousands)	Weight (thousand tonnes)	Biomass (million tonnes)	Food consumed (million tonnes) Krill	Squid	Fish
Baleen whales						
Blue	200	88	17.6	72	0.74	1.5
Fin	400	50	20.0	81	0.84	1.7
Humpback	100	27	2.7	11	1.11	0.2
Sei	75	19	1.4	6	0.06	0.1
Minke	200	7	1.4	20	0.20	0.4
Baleen total	975	—	43.1	190	2.00	3.9
Sperm whale	85	30	2.6	—	10.2	0.5

Table 2 Present whale stocks

Species	Population (thousands)	Weight (thousand tonnes)	Biomass (million tonnes)	Food consumed (million tonnes) Krill	Squid	Fish
Baleen whales						
Blue	10	83	0.83	3.4	0.04	0.07
Fin	84	48	4.03	16.4	0.17	0.34
Humpback	3	27	0.08	0.3	0.00	0.00
Sei	41	18	0.71	2.9	0.03	0.06
Minke	200	7	1.40	19.8	0.20	0.41
Baleen total	338	—	7.05	42.8	0.44	0.88
Sperm whale	43	27	1.16	—	4.63	0.24

Some of the consequences of the great differences between initial and present stocks are pursued below. A gross summary is that the pristine biomass density of baleen whales over the area of the krill zone was about $2.6\ \mathrm{g\ m^{-2}}$, while it is now $0.4\ \mathrm{g\ m^{-2}}$

Seals

The seal families probably originated about 30 million years ago in the northern hemisphere, and crossed the tropics along the west coast of South America during a cold period, to radiate into the Southern Ocean. There are now five species of 'true' seals south of the Antarctic Convergence—Weddell, leopard, crabeater, Ross and elephant—along with the Antarctic fur seal. Although there are fewer species in the Antarctic than in the Arctic (probably because the wide deep ocean has no geographical barriers to help speciation), the populations of individual species and the body sizes of their constituent members are larger (probably because food is more abundant). Of the roughly 30 million seals in the world, approximately half live in the Antarctic, where they constitute about 80% of the global biomass of seals.

The species achieve ecological segregation through a mixture of different habitats and different diets. The geographical aspect is set out schematically in Fig. 1, and the food preferences summarised in Table 3. The elephant and Ross seals are both deep-diving squid and fish eaters, but their breeding and feeding distributions have no overlap; the elephant seal breeds on land and is distributed about the Antarctic Convergence, while the Ross seal breeds on ice and is found near heavy pack ice. The fur and crabeater seals are primarily krill eaters; its name notwithstanding, the crabeater has dentition specialised to act as a krill-sieve, producing "perhaps the most complicated arrangement of cusps found in any living mammal" (Laws II). The fur seal breeds on land, and is restricted to the area between the pack ice edge and the Antarctic Convergence, while the crabeater breeds on ice and is restricted to the fringes of the pack ice belt. The leopard seal has a more varied diet (krill, fish, and seasonally penguins) and a wider range than the other seals, but it is primarily found at the fringes of the pack ice zone. The Weddell seal has adapted to fill a specialised niche—the inshore fast ice zone—where it winters under the ice, keeping breathing holes open with its teeth. It eats mainly fish.

The mating systems and patterns of sexual dimorphism correlate strongly with the ecology of the Antarctic seals (Stirling *Rapp. P.-v Renn. Cons. int. Explor. Mer.* **169**, 205; 1975). The polygynous Weddell has a social system intermediate between the highly polygynous and strongly dimorphic elephant and fur seals that breed on land, and the monogamous crabeater and other species that breed on shifting pack ice.

From Tables 1-3 it can be seen that the total biomass of Antarctic seals is less than 10% of the original biomass of large Antarctic whales, and less than half their present biomass. But the seals are present in Antarctic waters all year, and furthermore their metabolic rates and food consumption are relatively larger, because of their smaller body sizes. The consequent estimates of their total annual consumption of krill is about one-third that of the original stocks of baleen whales, and about 1.5 times that of the present whale stocks; seals have now surpassed whales as the major consumers of krill. Essentially all the krill eaten by seals is by crabeaters (to an excellent approximation all Antarctic seals are crabeater seals), who, by foraging at the margins of the pack ice, have relatively little direct overlap with whales.

Male sperm whales and both sexes of elephant seals eat mainly squid in the Antarctic, and, at present whale densities, they eat roughly equal amounts However, when the sperm whales are at peak abundance a large fraction of the elephant seal population is hauled out on land, fasting while breeding and moulting.

The average biomass density of krill-eating seals in summer is around 0.6 g m^{-2}, in regions where they compete with fish, birds and minke whales. The winter density is probably similar, but spread over a different area. Squid and fish-eating seals reach densities around 0.1 g m^{-2} when concentrated in summer, and around 0.03 g m^{-2} when dispersed in winter.

Birds and fish
Surveying a growing, but still limited, amount of information, Laws (II) concludes "the ecological separation of Antarctic birds appears to be determined by the summer distribution and breeding behaviour as they affect feeding". The segregation in the distribution and feeding ranges of some key species of penguins and other birds are shown in Fig. 1.

One example of the way the species sort themselves out is provided by three species of penguin that breed at Sigmy Island. The Adelie and chinstrap penguins feed mainly on krill, but they take different sized prey, either by active selection or by the Adelie feeding further from the rookery (or penguinery). The less common gentoo penguin feeds primarily on fish taken from the seabed.

Petrels and albatrosses also eat krill, squid and fish. In general, the lower latitude species such as the albatrosses are more dependent on squid and fish than the purely Antarctic species.

It is difficult to quantify the biomass and role in the ecosystem of Antarctic birds. Laws gives a rough estimate of 0.03 g m^{-2} for their biomass density when concentrated in the feeding zone in summer, and no more than 0.004 g m^{-2} when dispersed in winter.

Although the facts are not well established, the fish stocks in the Antarctic are probably much less than those in the Arctic. The main reason is that the Antarctic has a very narrow and relatively deep continental shelf, so that spawning sites for bottom-spawning fish are very limited. Some southern species have overcome this problem by spawning in coastal waters in the Subantarctic or on the continental shelf of Patagonia, and assuming a pelagic life as adults (when they feed on krill). In contrast with the north, where fish are the main vertebrate animals, in the Southern Ocean whales, seals and birds are more important.

Responses to the decrease in whales
By comparing Tables 1 and 2, it can be seen that the difference between the krill consumed annually by the original stocks of whales and that consumed today amounts to around 150 million tonnes.

What are the implications of this 'krill surplus' produced by whaling? General ecological theory suggests that, in the extreme case where there is no competition among whale species, nor between whales and other species, the 'surplus' will indeed appear as more krill. At the opposite extreme of high niche overlap among the vertebrate species, most of this 150 million tonnes of 'slack in the system' will be taken up (with time lags that may be measured in decades), causing shifts in the relative abundances of species. I think the reality is liable to lie between these two extremes.

Table 3 Seals

Species	Population (thousands)	Weight (thousand tonnes)	Biomass (million tonnes)	Food consumed (million tonnes) Krill	Squid	Fish
Elephant	600	0.50	0.30	—	4.5	1.5
Leopard	220	0.27	0.06	0.5	0.1	0.2
Weddell	730	0.25	0.18	—	0.5	2.2
Crabeater	14,800	0.19	2.87	63.2	1.3	2.0
Ross	220	0.17	0.04	0.1	0.6	0.2
Fur	200	0.05	0.01	0.1	0,1	0.1
	16,800	—	3.46	63.9	7.1	6.2

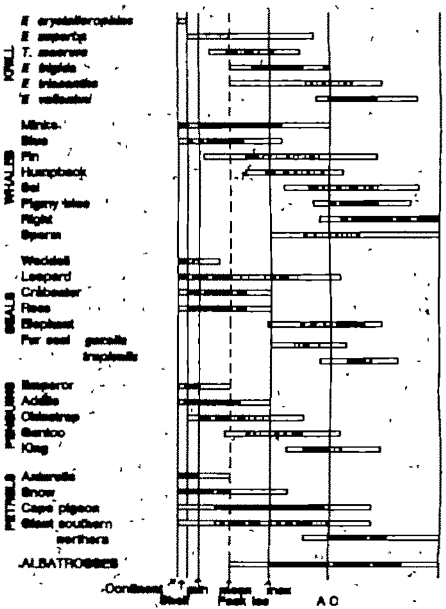


Fig. 1 A comparison of the zones occupied by selected species of krill, marine mammals and birds from the edge of the Antarctic Continent northward. The relative areas of the continental shelf, the minimum, mean and maximum area of pack ice, and of the region south of the Antarctic Convergence (A.C.) are as shown. Each species has a circumpolar distribution, and the average latitudinal range is as shown, with the shading denoting the regime of higher densities. (From Laws II).

Laws (I and II) summarises direct and indirect evidence of population increases in whales, seals and birds that may be attributed to the decline in baleen whale populations.

Increases in the numbers of king penguins seem greater than can be explained simply as recovery from the slaughter of the nineteenth century Population increases have also been shown for chinstrap, Adelie and gentoo penguins. It is noteworthy that the best-documented increases in these populations of krill-eating penguins are in the South Atlantic, where there is the greatest overlap between whales and penguins. In contrast, there is no indication of population increase for Adelie penguins on Ross Island over the past 50 years; interaction with whales is arguably small in the Ross Sea.

Payne (*Phil. Trans. Roy. Soc. Lond.* **B279**, 67; 1977) has established increases in the populations of the southern fur seal that clearly run ahead of simple recovery from over-exploitation. The fastest increase for this krill-feeding animal have taken place in the Scotia Arc, where its distribution overlaps that of baleen whales.

The sei whale has in recent years altered its migration patterns, arriving in the Antarctic earlier and penetrating further south. This could be due to reduced competition from blue, fin and humpback, but it could equally reflect some slow environmental change.

There is clear-cut evidence that pregnancy rates for blue, fin and sei whales have risen, and that the mean ages at sexual maturity have fallen, since intensive whaling was resumed after World War II. For the fin, the age at maturity was steady around 10 years from 1910–30, and has declined to 5–6 years in the most recent age classes. This suggests, again indirectly, increased food abundance. Similar evidence has been adduced for the crabeater seal. In the Antarctic Peninsular, the mean age at sexual maturity for this krill-eater was 4 years until the whaling zone known as the 'Sanctuary' was reopened; since then, the age has fallen to 2.5 years.

The question of the rate at which minke whale stocks are increasing, presumably in response to the effective disappearance of its close competitor the blue (see Fig. 1), is currently the subject of some dispute (IWC, *Rep. int. Whal. Commn.* **28,** 55; 1978).

Laws (II) concludes, "the baleen whales probably remain the most important vertebrate group in their effect on the antarctic ecosystem. Their stocks are likely to stabilise or slowly increase with realistic scientific management, but it is uncertain whether their original abundance will be regained even if commercial whaling ceases". ☐

X-ray absorption spectroscopy

from C. David Garner

ALTHOUGH the existence of fine structure on X-ray absorption edges has been well known for more than 40 years, it is only recently that such data have been shown to provide reliable information concerning the electronic structure and the atomic arrangement about specific atoms in simple or complex materials. This transformation has been made possible by exploitation of the intense continuum of electromagnetic radiation, extending to wavelengths below 1 Å, available from high-energy electron or positron accelerators as a natural consequence of maintaining the charged particles in a circular trajectory. This radiation has a number of unique properties (Rowe & Weaver *Sci. Amer.* **236,** 32; 1977) and is generally known as synchrotron radiation.

The structural information derives from an accurate measurement of the variations in the X-ray absorption coefficient at and above absorption edge(s) of the element(s) of interest (Sayers, Lytle & Stern, *Adv. X-Ray Anal.*, **13,** 248; 1970: Stern *Sci. Amer.* **234,** 96; 1976). Present monochromator design and other limitations effectively restrict studies to the *K*-edges of the elements Ca–Mo and the *L*-edges of elements Ag–U. It is convenient to discuss the potential information contained within the X-ray absorption spectrum as three topics. (1) The actual energy of a particular absorption edge of an element depends on the oxidation state and the nature of the immediate chemical environment of that element. Typically, one unit increase of oxidation state increases the position of the edge by 1–3 eV. (2) The excitation of the core electron into the continuum may be incorporated with transitions from the inner core level to outer bound levels. These internal excitations are usually evident as discontinuities on the primary absorption edge (edge structure) and they can provide information about the electron structure at the absorbing atom in the material of interest. (3) The emitted photoelectron can be considered as a spherical wave radiating from the absorbing atom. This wave will be partially reflected by the electron clouds of the atoms surrounding the absorber; the resultant interference between outgoing and back-reflected waves is manifest as a modulation of the X-ray absorption coefficient at energies greater than that of the absorption edge. This extended fine structure (EXAFS) thus contains information about the number and the type of atoms which surround the absorber and the corresponding interatomic distances. Many of the theoretical problems associated with the interpretation of EXAFS data now seem to have been overcome (Lee & Pendry *Phys. Rev. B* **11,** 2795; 1975: Teo, Lee *et al.*, *J. Amer. Chem. Soc.* **99,** 3854; 1977) and the validity of the structural predictions have been confirmed in a number of studies on materials of a known structure. (Hodgson *et al. J. Amer. Chem. Soc.* **100,** 2748; 1978).

X-ray absorption spectroscopy offers two particular advantages for the study of metal ions in biological systems. First, there is no requirement that the material of interest be crystalline, in principle gases, liquids, glasses and amorphous solids can be investigated. Second, the measurements are specific to the element of interest which may be present at a low concentration, say <1 atom in 10^4. Several investigations of the coordination about a metal ion in a metalloprotein have now been accomplished and the precision of the bond length data, which typically extended to $\sim$3 Å from the primary absorber, can be $\pm$0.02 Å. A unique and valuable insight into the nature of the molybdenum centre of the nitrogenases, has been provided by the measurement and interpretation of EXAFS data for the Mo–Fe protein of *A. vinelandii* and *C. pasteurianum* and the FeMo cofactor of the former (Hodgson *et al. J. Amer. Chem. Soc.* **100,** 3398, 3814; 1978). EXAFS studies for *P. aerogenes* rubredoxin (Shulman *et al. Proc. natn. Acad. Sci. U.S.A.* **72,** 4003; 1975) indicated that all four Fe–S bonds were of equal length, in contrast to the initial prediction of three long and one short Fe–S bond from an X-ray crystallographic structure determination. Subsequent revision of the latter led to a concordance between the two approaches. A similar unanimity exists between the EXAFS and X-ray diffraction studies accomplished for the copper centre of the 'blue' proteins (Hodgson *et al. Proc. natn. Acad. Sci. U.S.A.* **75,** 4069; 1978; Freeman *et al. Nature,* **272,** 319; 1978).

However, the EXAFS determinations of the Fe–N distances in oxy and deoxyhaemoglobin (Eisenberger *et al. Proc. natn. Acad. Sci. U.S.A.* **73,** 491; 1976) have indicated that the iron atom moves much less than the 0.7 Å away from the haem plane suggested from the Hoard–Perutz model. It is thus suggested that the cooperativity between the haem centres does not originate from a significant displacement of the iron atom upon oxygenation.

The paper by Labhardt and Yuen in this issue of *Nature* (page 154) describes the results of an X-ray absorption spectroscopic study of the iron centre of cytochrome *c*. In contrast to the results of an earlier study (Shulman *et al. Proc. natn. Acad. Sci. U.S.A.* **73,** 1384; 1976), a shift in the iron *K*-edge position and the association edge structure of $\sim$1.5 eV was observed on oxidation. The sense and magnitude of the shift are consistent with a redox process which is localised at the iron centre. Furthermore, the EXAFS details indicate that the lengths of bonds involving the iron atom do not change on oxidation (although there is no indication in this paper that these details are consistent with those expected from the studies of Dickerson *et al. J. biol. Chem.* **248,** 5234; 1973). Therefore, the two principal conclusions from this study are compatible with a view that, as expected from Frank–Condon considerations, electron transfer metallo-

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proteins contain centres for which the redox change, although primarily located on the metal centre, does not require nor lead to a change in the geometry of the immediate environment of the metal. The EXAFS results for cytochrome *c* and haemoglobin indicate that the conformational changes in the peptide, which register the change in state of the metal centre, almost certainly do not originate as a result of changes in the iron-ligand bond lengths. Labhardt and Yuen suggest that the conformational transition may be initiated by a change in the amplitude of certain low frequency vibrations. This is possible, although, in contrast to their specifications, it seems more likely that the reduced iron centre would lead to a more flexible structure than its oxidised counterpart. Thus the weaker metal–ligand interactions expected for the reduced centre would be expected to result in vibrational amplitudes, for example, for metal–ligand bending modes which, although corresponding to approximately the same average interatomic distances, would be larger than obtained for the corresponding oxidised system at the same temperature.

A real assessment of the contribution of X-ray absorption spectroscopy must await a wider and more detailed series of studies than accomplished thus far. Certain of the present limitations which restrict the range of systems which may be studied and potential interpretive ambiguities will undoubtably be reduced in the near future. However, there seems to be no prospect of obtaining angular as well as radial structural data. Nevertheless, there is no doubt that the validity of this technique is well established and that it offers unique opportunities for the detailed investigations in a wide range of systems. The impact on inorganic biochemistry should be particularly significant. ☐

Coated vesicles

from M. Geisow

GLUTS or shortages of the membranes which enclose cells and their constituent organelles arise under some circumstances. For example, during endocytosis, the plasma membrane of cells is progressively depleted as the cytoplasm becomes filled with endocytotic vesicles. Conversely, following the stimulated release of cell products, excess membrane accumulates at the cell surface at the expense of intracellular pools. In both cases a recovery system is necessary. Reversion to a resting state is achieved in many exocytotic systems by the interiorisation of excess plasma membrane. Vesicles return through the cytoplasm either to subcellular membranes or to lysosomes where they are degraded. Whilst little is known about the nature and composition of such vesicles, recent progress has been made with one class of vesicles with a curious polyhedral 'exoskeleton' which encloses their membranes. This structure has given them the name 'coated vesicles'.

Coated vesicles have been recognised for many years. They were first observed in association with embryonic nutrition, where invaginations of the oocyte membranes were seen to take up food from the external environment into vesicles with a 'bristle coat'. Subsequently many workers have noted this feature in both endocytotic and exocytotic profiles. Palade and his colleagues reported coated vesicles in the Golgi region of secreting cells. Franke *et al.* (*J. Cell Biol.* **69**, 173; 1976) identified an exported product of mammary gland (casein) in coated secretory vesicles.

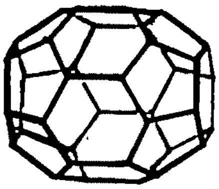

Fig. 1 One structure assigned by Crowther *et al.* to vesicle coats.

Later studies have suggested that the coated vesicle might represent a device for recycling cell membrane. In frog neuromuscular junction Heuser and Reese (*J. Cell Biol.* **57**, 315; 1978) described the sequential fusion of synaptic vesicles with junctional membrane followed by the pinocytosis of presynaptic membrane as coated pits which became coated vesicles. The earlier work of Holtzman *et al.* (*Science* **173**, 733; 1971) with lobster neuromuscular junctions was supported by these findings, although the latter article reported few coated vesicles.

Barbara Pearse has devised a procedure for isolating coated vesicles from various tissues (*J. molec. Biol.* **97**, 93; 1975). Their lattice-like coats are composed predominantly of one type of 180,000 molecular weight polypeptide which she has called clathrin. Several analyses of the coat morphology have been made (for example Kaneski & Kadoki *J. Cell Biol.* **42**, 202; 1969). This and more recent studies (Crowther *et al. J. molec. Biol.* **103**, 785; 1976; Woods *et al. J. Cell Sci.* **30**, 87; 1978) indicate that the clathrin lattice is formed from trimeric associations of clathrin to give hexagonal and pentagonal units (Fig. 1). Alteration in the number of hexagonal units allows the size of the enclosed vesicles to vary without distortion of the lattice.

Peptide maps of clathrin prepared from different species indicate that the protein is strongly conserved (Pearse *Proc. natn. Acad. Sci. U.S.A.* **73**, 1255; 1976). There are also close similarities in the more minor components of coated vesicles isolated from different tissues (Pearse *J. molec. Biol.* **126**, 803, 1978). Pearse suggests that some of these might have a structural role, perhaps relating to the vertices of the polyhedral coat. However, the number of polypeptides obtained from the isolated coated vesicles is small, which is surprising in view of the ultrastructural evidence that they participate in endocytotic and exocytotic transport. Both Pearse and also Blitz *et al.* (*J Cell Biol* **75**, 135; 1977) report polypeptides of 100,000 and 55,000 molecular weight. The latter workers consider that these molecules resemble the major constituents of sarcoplasmic reticulum. They therefore infer that the molecules belong to the transported vesicle rather than to its polyhedral coat. The larger polypeptide in particular appears to be a calcium-transporting ATPase and cross reacts with antisera to the authentic sarcoplasmic reticulum enzyme. It is notable that intracellular and pericellular membrane pools for which the coated vesicle may act as a go-between, contain substantial amounts of calcium-stimulated ATPase (for example, Golgi membranes: Hodson *J Cell Biol.* **30**, 117; 1978), endoplasmic reticulum: Macdonald *et al J. biol Chem.* **253**, 3504; 1978) More work is needed, however, to establish firmly the nature of the components in coated vesicles.

Other intrinsic membrane proteins may be carried by the enclosed vesicle. For example, Gorden *et al* (*Proc natn. Acad Sci. U.S.A.* **75**, 5025; 1978) in a study of the binding of ^{125}I-epidermal growth factor (EGF) to human fibroblasts found that one-third of the specifically bound label rapidly became associated with bristle-coated areas of the plasma membrane (representing less than 2% of the total cell surface) Label and membrane were pinocytosed to form a coated vesicle. It seems plausible that the EGF receptor was also lost in this way, as prolonged stimulation depleted the surface receptors.

Many questions remain unanswered about coated vesicles in particular and

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membrane transport in general. Understanding the control of the assembly, disassembly and destination of coated vesicles now provides an experimental challenge—but why is the cage structure necessary at all? One suggestion has been made that the clathrin physically pinches off a membrane vesicle and at the same time controls the size of the final structure. Another suggestion might be made here. It is widely held that cellular membranes retain their identity with respect to membrane components, even when extensive membrane transport occurs. Is it possible that the closely-applied clathrin molecules provide a 'filter' for some categories of membrane protein, preventing their equilibration between pericellular and subcellular pools? ☐

Prospects for solar energy

from Elizabeth Michelson

THE construction and monitoring of both highly-insulated experimental solar heated houses and solar installations in conventional housing is becoming quite widespread. At a recent meeting held in London* G. O. G. Löf reported on experimental houses that have been built at Colorado State University to compare different types of solar heating systems. Air-circulating systems, with rock storage, were found to supply much the same amount of energy as flat-plate water collectors but were preferable on grounds of cost, durability and lack of problems of corrosion or damage from leakage. In the UK the Building Research Establishment at Watford is to install identical solar heating systems in 80 existing typical 3-bedroomed houses, in order to give a distribution of total daily hot water use, S. J. Leach reported. The ratio of panel area to total daily hot water use and the hot water delivery temperature are the main parameters determining the system performance (B. J. Brinkworth, University College, Cardiff).

There were many shades of opinion on the economic feasibility of solar domestic heating, largely because it rests on speculation as to fuel costs and the general economic situation in the near future. Leach held that a system could only be regarded as economic if it were so now but Brinkworth pointed out that insufficient work has yet been done on the durability of systems, and that this has to be taken into account if the economic viability is to be determined in any set of circumstances. In general, it was agreed, solar water heating is certainly practicable in the UK.

H. Hörster (Philips, Aachen) concluded from theoretical studies that space heating was uneconomic at present in North European or UK conditions. An average house would cost some £2,500 to convert it to the high standard of insulation (as in the Swedish building regulations) needed before space heating is economically feasible. In the South of France however, solar space heating is a good prospect even in the normal house. Between sixty and seventy per cent of US homes already have air-circulating central heating systems and Löf considered that air collectors for space heating, which are presently less common, will become competitive in the near future.

Solar ponds

A solar pond is a water-filled pond, 1-2 m deep, with a blackened bottom for greater heat absorption and a gradually increasing salt concentration towards the base to eliminate convection, which is the main cause of heat loss (H. Tabor, The Hebrew University, Jerusalem). The hot water remains at the bottom from which it is removed as required. Solar ponds tackle several of the major problems associated with other types of collectors; they can easily cover a large area and act as the store at the same time. In Israel, a solar pond will collect about 20% of the incident radiation and Tabor estimated that 200 km^2 of solar ponds would supply all of Israel's current demand for electricity. However, owing to the low ratio of winter to summer radiation, solar ponds are unsuited to high latitudes.

Solar cells

Solar or photovoltaic cells convert light directly into electricity. Of these, single-crystal silicon cells are so far the only reliable and durable ones and have been used for many years in space and for the generation of small quantities of electricity in remote areas (H. Durand, Commissariat à l'Energie Solaire, Paris). Heterojunction cells, in which the semiconductor is made up of two different materials, have the advantage of requiring a much lower-temperature manufacturing process, but have not yet equalled the efficiency and durability of silicon cells (S. Wagner, Solar Energy Research Institute, Golden, Colorado). Optimism for the extensive use of solar cells in the future is witnessed by the passing of the US Photovoltaic Bill allocating $1·5 billion over the next 10 years, $125 million for the present year (Durand). The corresponding present annual budget for Europe is £2·5 million for France and £2 million for West Germany. However, the attractive prospect of direct electricity has cost as one of its chief drawbacks. At present, 1 kW costs between £20,000 and £25,000; improved technology and mass production could reduce this by a factor of thirty. At the same time, the present efficiency of about 16% should not fall below 10% for the costs of encapsulation and installation to remain covered. The US hopes to add solar-generated electricity to its national grid, which can absorb random inputs of ± 15%, since large storage facilities are not feasible.

Energy from biomass

The two most interesting end-products of the action of microorganisms on organic material are alcohol (ethanol) and methane (R. C. Righelato, University of Reading). The production of ethanol by fermentation is very costly because a dilute aqueous environment is required for the reaction and a large quantity of energy, comparable with the energy content of the ethanol, is required to recover the latter from solution. Improvements can be made by finding a method for recovering the alcohol which is less energy demanding than distillation, or using less water initially. Some yeasts can act in concentrations of up to 20% if only for a short time. Ethanol has been produced as a fuel in Brazil, though it is unlikely to make much contribution in the UK.

Gaseous products such as methane emerge from the solution unaided. The production of methane from sewage is more efficient than fermentation, requires less energy input and processes all the material including the cellulose. Methane production has already shown itself to be very practical and is particularly suited to small installations on farms.

As solar collectors, plants have some considerable advantages over man-made collectors. They have large surface areas, built-in storage and an enormous diversity, adapted to almost all conditions (N. K. Boardman, CSIRO, Dickson, Australia). However, the average efficiency of conversion of solar energy is only about 1%. The yield can be increased by the use of fertilisers, coppicing and short rotation crops, though the long-term environmental consequences of these are as yet unknown. Crop residues should also be utilised, but some must remain on the soil to inhibit wind and water erosion and supply nutrients. The crop can either be used as a dry fuel (such as timber) or converted to

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*A discussion meeting on Solar Energy was held on 15–16 November, 1978 at the Royal Society, London.

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ethanol, methane or oil with conversion efficiencies of 20–44% for gases and 58% for oil. Boardman estimated that such liquid fuels could contribute 47% of Australia's transport fuel requirements, one-third originating in crop residue and two-thirds in new fuel crops. Similarly good possibilities exist in Brazil and New Zealand. However, biomass is unlikely to make a significant contribution to the energy requirements of small densely populated countries.

The overall impression was one of optimism, based not on the all-embracing potential of any one method, but on the diversity of methods of harnessing solar energy which, given a rational energy consumption, can make some contribution in almost every climate. □

From sea level to heat flow

from Peter J. Smith

When hot material of mantle origin spreads away from an oceanic ridge zone it cools and contracts. Both theory and observation indicate that the seafloor subsidence thereby produced is proportional to the square root of time, from which it follows that a fast-spreading ridge will have a greater volume than a slow-spreading one. It follows from that, in turn, that, assuming the volume of seawater and the radius of the Earth to remain constant, the sea level will rise during a period of fast spreading. So could variations in ridge spreading rate be responsible for some or all of the fluctuations in sea level that have been known now for more than a hundred years? Hays and Pitman (*Nature* **246**, 18; 1973) thought so and thus added to the large number of explanations previously suggested for changing sea level (glaciation, continental volume changes, Earth's radius changes).

Now Turcotte and Burke (*Earth Planet. Sci. Lett.* **41**, 341; 1978) have taken the logic of this model one stage further and, assuming sea level fluctuations indeed to be indicative of changes in ridge volume, have related changes in sea level to changes in ridge heat flow. The sea level data used were obtained indirectly from Hallam (*Nature* **269**, 769; 1977), who estimated the percentage flooding of North America and the Soviet Union over the past 520 million years from palaeogeographic maps, and from Vail *et al.* (*Am. Assoc. Pet. Geol. Mem.* **26**, 83; 1978), who determined relative sea levels directly for the past 600 million years using a combination of seismology and stratigraphy to detect the onlap of coastal deposits in maritime sequences. The two data sets suggested remarkably similar sea level trends, thereby giving some confidence in the apparent changes in water volume, and hence the supposed changes in ridge volume derived from them, taking into account isostatic responses.

Relating ridge volume to ridge heat flow is a more complex operation in the mathematical sense but can be done using thermal boundary layer theory. Expressing the ridge heat flow as a proportion of total world heat flow is rather simpler, however, although the number of assumptions required (for example, that the Earth's heat is entirely due to uranium, thorium and potassium in the Earth) begins to mount alarmingly. Given the number of assumptions involved and the considerable uncertainty in many of the numerical values used, Turcotte and Burke are cautious in interpretation. Nevertheless they feel justified in coming to two tentative general conclusions—first, that the proportion of the Earth's heat dissipation occurring in ridge zones has generally declined slowly over the past 600 million years much in accord with the decline in heat generation over that period, and, second, that there have been times during the Phanerozoic when the proportion of the Earth's head dissipation occurring in ridge zones, at present 28 per cent, has risen as high as 50 per cent.

According to the interlocking model thus far developed, these ridge heat flow 'highs' coincide, of course, with sea level maxima and hence with the times of the most extensive continental flooding—and the latter occurred about 80 and 400–500 million years ago. The 600 million years of observation is far too short to support any suggestion that these figures indicate cyclic behaviour with a periodicity of about 300 million years, although it is interesting to note that 300 million years is about equal to the turnover time of a mantle convection cell. Moreover, if sea level and ridge heat flow maxima are indeed causally related, both would be expected to be related to convective behaviour, for heat flow variations almost certainly are.

And that leads to another thought. High ridge heat flows correspond with ridge volume maxima which coincide with fast ridge spreading. In turn, this must coincide with increases in the amount of mantle material upwelling at ridges which (assuming an Earth of constant radius) must be connected with increases in the rate of lithospheric consumption at subduction zones. As orogenic behaviour is mainly associated with plate consumption, the implication here is that orogenic processes are also episodic, reaching greatest intensity when the sea level is highest. If it be true, as Turcotte and Burke argue, that the widespread destruction of rocks and structures during mountain building obscures much of the evidence of how and when mountains formed, sea level studies may turn out to be useful in determining the precise temporal variations in orogenic activity. □

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NATURE

A hundred years ago

I HAVE just read in *Nature* (vol. xix, p. 182) an account of a strange electrical phenomenon observed at Teignmouth. In connection with it the following incident may be of some interest:—When in Switzerland, not long since, I made with some friends the ascent of Monte Rosa. The weather was unsettled, and on gaining the summit we saw a thunderstorm advancing in our direction from the Italian valleys, and not wishing to turn ourselves into lightning-conductors we deemed it wise to retire from the summit. We had retreated a very short distance along the *arête* when the storm-clouds swept up upon us; the fine snow fell so thick that we could hardly see one another, and we were all suddenly attracted by a peculiar ticking or fizzing from our hair; when I held up my axe the ticking was most distinctly heard from the top of it. The thunder ceased, and we felt that we were acting as points, through which the ground electricity was flowing off into the cloud; if it had been dark, the bluish light observed at Teignmouth might have been visible.

As at Teignmouth, so on Monte Rosa; it was freezing hard when the phenomenon was observed.

W. S. GREEN

Alta Terrace
Monkstown, Cork
From *Nature* **19**, 9 Jan., 220; 1879.

Erratum

In the article 'Discrepancies in claims for endothermy in therapsids and dinosaurs' (*News and Views* **276**, 757; 1978) the last eight lines of paragraph 1 should read: "As usual some disputed issues reflect different individual views of identical evidence, but one of Bakker's later arguments involves a serious factual error, unless many other authors are mistaken, while some of the arguments used in different papers are inconsistent or contradictory."

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review article

Multiple receptors for dopamine

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Pharmacological and biochemical criteria can be used to separate those dopamine receptors which are linked to the enzyme adenylyl cyclase and those which are not.

BEFORE discussing the existence of multiple receptor mechanisms for dopamine, some general comments about the definition of receptors are appropriate. The concept of a 'receptor' for a substance arises from the ability of tissue both to recognise and respond to minute quantities of a substance in some precise way. Receptors may be defined according to many different criteria, but it is most profitable to identify the endogenous physiological agent which normally interacts with the receptor. The next stage of classification is based on a combination of pharmacology (that is, identification of drugs which selectively mimic or antagonise the physiological agent) with biochemistry, anatomy and physiology. This second level of categorisation is most useful when it generates the least number of subgroups; however, within a given category, further division is possible into a hierarchy of descending levels (for example, nicotinic cholinergic receptors are further subdivided into ganglionic and neuromuscular receptors). There are many precedents for the existence of multiple classes of receptors for a given biological molecule. Thus, acetylcholine interacts with nicotinic and muscarinic receptors, histamine with H_1 and H_2 receptors, and catecholamines with α- and β-adrenergic receptors.

Dopamine receptors can be characterised by various criteria. In earlier discussions, dopaminergic receptors were classified on the basis of physiological[1] or pharmacological and biochemical[2,3] criteria; alternatively a dynamic equilibrium of a single dopamine receptor fluctuating between two states or configurations has been postulated[4]. The demonstration of a dopamine-sensitive adenylyl cyclase not only provided an *in vitro* model to study the properties of a dopamine receptor but also suggested a mechanism for the generation of the physiological response to this amine[5]. However, not all dopamine receptors have properties similar to those of the receptor linked to adenylyl cyclase. Furthermore, in some tissues, a dopamine-stimulated accumulation of cyclic AMP does not seem to be involved in the generation of the physiological response to dopamine.

Dopamine receptors which increase cyclic AMP synthesis

One physiological action of dopamine is to increase the synthesis of cyclic AMP by the stimulation of a specific dopamine-sensitive adenylyl cyclase[6,7] (for review see ref. 8). The dopamine-sensitive adenylyl cyclase is widely distributed, particularly in neural tissue ranging from gastropods[9] and insects[10] to human brain[11]. The demonstration of a dopamine-sensitive adenylyl cyclase in cell-free preparations of caudate nucleus[6] raised the possibility that the postsynaptic dopamine receptor in this region of the brain could regulate the enzyme. This concept provided the impetus for a detailed characterisation of the dopamine receptor associated with the striatal enzyme; the procedures used to define this receptor involved the use of dopamine itself, analogues of dopamine, and synthetic drugs which stimulate or block dopamine receptors.

Although many structural analogues of dopamine have been tested for their effects upon the striatal adenylyl cyclase, no catecholamine is more potent than dopamine itself. The only catechol-containing molecule which is equipotent with dopamine is epinine (*N*-methyldopamine); any other substitutions on the dopamine molecule produce a loss of agonist activity[12-14]. Summaries of these structure-activity studies have been published previously[8,15]. A number of analogues, which possess dopamine-like activity *in vivo*, have also been shown to mimic the ability of dopamine to increase adenylyl cyclase activity. The semirigid dopamine analogue, 6,7 dihydroxy-1,2,3,4-tetrahydronapthalene (A-6, 7-DTN), is equipotent with dopamine as an *in vitro* agonist[12]. Other structural analogues give less consistent results. Apomorphine is 'classically' recognised to mimic the effects of dopamine[16], but when tested on the striatal enzyme system, this molecule is only a partial agonist[6]; it can antagonise dopamine receptors both in cell-free homogenates[6] and in preparations of intact cells[17].

Antagonists have also been used to define the properties of the dopamine receptor which regulates striatal adenylyl cyclase. Classic antagonists of α- and β-adrenergic receptors are relatively ineffective as antagonists of dopamine[6], but antipsychotic drugs are potent, competitive antagonists[6,11,18,19]. Several dopamine antagonists exist as optical or stereoisomers; the stereoisomers of butaclamol and *cis*- and *trans*-flupenthixol are examples. The clinically active compounds (+)butaclamol and *cis*-flupenthixol are potent dopamine antagonists while the clinically and pharmacologically inactive compounds (−)butaclamol and *trans*-flupenthixol are less potent as dopamine antagonists[18,20,21].

The relative imprecision of the experimental measures of *in vivo* dopaminergic function (for example, turning and stereotypic behaviour in rats, neurological or psychiatric changes in man) preclude an exact definition of the role of dopamine-sensitive adenylyl cyclase in the brain; although dopamine and cyclic AMP have been implicated in electrophysiological events in mammalian superior cervical ganglia[22,23] and in a *Helix* ganglion[9]. However, activation of dopamine receptors of isolated cells of the bovine parathyroid is followed by the accumulation of cyclic AMP and the release of parathyroid hormone[17]. This indicates that tissue-specific responses to dopamine can be mediated by the dopamine-sensitive adenylyl cyclase.

Dopamine receptors which do not increase cyclic AMP synthesis

Other physiological responses to dopamine seem not to involve either the stimulation of an adenylyl cyclase or the accumulation of intracellular cyclic AMP. An example is the dopamine-induced inhibition of prolactin release from mammotrophs.

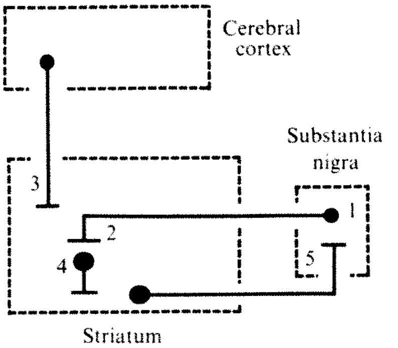

Fig. 1 A schematic representation of the neurones in the nigrostriatal axis which contain dopamine receptors. The dopaminergic neurones contain autoreceptors which regulate either electrical firing of these cells in the substantia nigra (site 1) or tyrosine hydroxylase activity (site 2) on the terminals. Cortical neurones which project to the striatum possess binding sites for radiolabelled haloperidol which have been identified as dopamine receptors (site 3). Neurones intrinsic to the caudate contain the dopamine-sensitive adenylyl cyclase activity (site 4). Finally, terminals of striatal neurones which project to the nigra also contain a dopamine-sensitive adenylyl cyclase (site 5). The dopamine receptors at sites 1–3 have not been associated with adenylyl cyclase activity; only the receptors at sites 4 and 5 regulate this enzyme activity (for references see text).

The synthesis and release of prolactin are under a predominantly inhibitory hypothalamic influence: dopamine in the median eminence functions as an endogenous hypothalamic factor which inhibits prolactin release (for comprehensive reviews see refs 24, 25). The anatomical, physiological, pharmacological and biochemical evidence that demonstrates the presence of receptors for dopamine on the mammotrophs includes the following evidence: (1) Catecholamines, including dopamine, can inhibit the release of prolactin *in vivo* and *in vitro*[26,27]. (2) Dopaminergic agonists, such as apomorphine[28–30] can mimic the inhibitory effect of dopamine. (3) The effects of dopamine can be blocked by several selective dopaminergic antagonists[28,30]. (4) Radiolabelled agonists and antagonists of this receptor bind with high affinity to stereospecific sites that have properties similar to those of the dopamine receptor on the mammotrophs[30–32].

There is no evidence that a dopamine-stimulated accumulation of cyclic AMP can account for the inhibitory effect of dopamine on prolactin release. Cholera toxin, the nonspecific activator of adenylyl cyclase (see ref. 33 for review) has been added to cells of the anterior pituitary or cloned tumour cells derived from the anterior pituitary. Cholera toxin either stimulates[34] or has no effect on the release and synthesis of prolactin (M. Thorner, personal communication); it never decreases prolactin release. Furthermore, dopamine inhibits the adenylyl cyclase from prolactin secreting human pituitary adenomas[35].

A second example of a dopamine receptor which does not stimulate adenylyl cyclase activity is the dopamine receptor (autoreceptor) on the dopaminergic nigro-neostriatal neurones, which are found on presynaptic terminals in the striatum and also on the cell bodies of the same neurones in the substantia nigra[36,37]. Although a dopamine-sensitive adenylyl cyclase has been identified in the substantia nigra[38,39] as well as the striatum[6], the enzyme occurs on cellular elements other than the dopaminergic neurones. Lesions in the nigra induced by 6-hydroxydopamine (which selectively destroys the dopaminergic neurones) do not alter the activity of dopamine-sensitive adenylyl cyclase within either the nigra[39] or the striatum[40]. Conversely, intrastriatal injection of kainic acid, a rigid glutamic acid analogue, destroys neuronal cell bodies in the striatum but spares the dopaminergic nerve terminals; this procedure causes a substantial loss of striatal dopamine-sensitive adenylyl cyclase activity but does not diminish the content of dopamine[41,42]. Thus neither the autoreceptors upon cell bodies in the nigra nor the autoreceptors on the terminals in the striatum appear to be associated with a dopamine-sensitive adenylyl cyclase.

Pharmacological separation of dopamine receptors

Pharmacological evidence indicates the existence of several classes of dopamine receptors. Several antagonists are not equally effective upon different dopamine receptors. For example, the antipsychotic drugs, molindone and sulpiride, and the antiemetic drug, metoclopramide, are dopamine antagonists when tested in the anterior pituitary[43–45] or the brain[47,75]. However, neither metoclopramide nor sulpiride blocks striatal dopamine-sensitive adenylyl cyclase[3,46,75]. Molindone is, at most, a weak antagonist of this enzyme (B. K. Krueger and P. Greengard, personal communication; M. Munemeura and J. W. Kebabian, unpublished observation).

Other compounds are capable of a more limited discrimination between different dopamine receptors. Dopamine itself displays a considerable range of potency at its receptors in different tissues. Nanomolar concentrations of dopamine elicit a response from either the anterior pituitary ($K_a = 35$ nM) or the presynaptic dopaminergic receptors on the sympathetic terminals in the artery of the rabbit ear ($K_a = 37$ nM)[30,48]. The presynaptic autoreceptor on the terminals of the nigro-neostriatal neurones is stimulated by somewhat higher concentrations of dopamine[49]. In contrast, dopamine receptors linked to adenylyl cyclase are stimulated by micromolar concentrations of dopamine. This observation has been made repeatedly in cell-free homogenates of neural tissue derived from species as divergent as the cockroach ($K_a = 2$ μM)[10] and man ($K_a = 6$ μM). Micromolar concentrations of dopamine are also necessary for the accumulation of cyclic AMP in preparations of tissue containing intact cells[5]. Finally, micromolar concentrations of dopamine are required either to stimulate cyclic AMP accumulation or to elicit release of parathyroid hormone in preparations of the bovine parathyroid[17]. Thus, the relative potency of dopamine can be the basis of a categorisation of dopamine receptors.

Apomorphine is another compound with selective activity on different dopamine receptors. In many tissues, apomorphine mimics the effects of dopamine; both dopamine ($K_a = 35$ nM) and apomorphine ($K_a = 3$ nM) are potent activators of the pituitary dopamine receptor[30]. Apomorphine ($K_a = 44$ nM) also mimics the effect of dopamine ($K_a = 37$ nM) on the sympathetic presynaptic receptors of the rabbit ear artery[48]; the potency of apomorphine ($K_a = 300$ nM) and dopamine ($K_a = 320$ nM) on the autoreceptor on the terminals of the nigro-neostriatal neurones are similar[49]. In contrast, in several tissues where dopamine receptors are linked to the enzyme adenylyl cyclase, apomorphine displays activity as a dopamine antagonist. In the striatal dopamine-sensitive adenylyl cyclase system, apomorphine has actions as both an agonist (at low concentrations) and an antagonist (at higher concentrations)[6]; similar observations have been made in kidney vasculature[50]. Apomorphine antagonises the dopamine-stimulated accumulation of cyclic AMP in the bovine parathyroid; the compound has no agonist activity on these cells[17]. Thus, the different pharmacological actions of apomorphine imply the occurrence of more than one type of receptor.

Several ergot derivatives can also distinguish different dopamine receptors. The dopaminergic ergots, such as bromocriptine, lisuride and lergotrile, are extremely potent agonists of the dopamine receptor of the mammotrophs. When tested on isolated cells of the anterior pituitary, bromocriptine has a K_a of 2.9 nM, which is approximately 10-fold more potent than dopamine itself ($K_a = 35$ nM)[30]. Extremely high concentrations of the ergots can cause accumulation of cyclic AMP in some tissues, thereby mimicking the action of dopamine. For example, bromocriptine, like dopamine, causes an accumulation of cyclic AMP in the rabbit retina, but this effect requires a 33,000-fold higher concentration than is needed to inhibit prolactin release[51]. Of greater interest is the ability of such ergots to block the striatal dopamine receptor linked to adenylyl cyclase. This antagonist activity, which is unanticipated in view of the action of such compounds on the anterior pituitary, is seen in both intact and homogenised preparations of striatal tissue[52,53]. When tested in the cell-free striatal homogenate, two dopaminergic ergots, lisuride[54] and lergotrile[55,56] are devoid of agonist activity; both of these compounds are antagonists of the dopamine receptor. These differences in potency and action of the dopaminergic ergots point to differences between the dopamine receptors found in the pituitary and dopamine receptors associated with adenylyl cyclase in the striatum.

Table 1 Criteria for the classification of dopamine receptors

Name*	D-1	D-2
Cyclase linkage	Yes	No
Location of prototype receptor	Bovine parathyroid	Mammotroph of anterior pituitary
Dopamine	Agonist (μmolar potency)	Agonist (nmolar potency)
Apomorphine	Partial agonist or antagonist	Agonist (nmolar potency)
Dopaminergic ergots	Potent antagonist (nmolar potency) Weak agonist (μmolar potency)	Agonist (nmolar potency)
Selective antagonist	None known as yet	Metoclopramide sulpiride
Radiolabelled ligand	*cis*-flupenthixol†	Dihydroergocryptine

* Previously[65], we have designated the two categories of dopamine receptors as 'α-dopaminergic' and 'β-dopaminergic'. This has led to confusion with the α and β adrenoreceptors. The new designations should prevent further confusion.

† Radiolabelled *cis*-flupenthixol can be used as a ligand specific for the dopamine receptor linked to adenylyl cyclase in the rat striatum[64]. Its affinity for the dopamine receptor in the anterior pituitary has not been measured.

Although antipsychotic agents are potent antagonists of dopamine receptors in many tissues, these drugs are of only limited use in discriminating between the different types of dopamine receptors. Various chemically distinct antipsychotic drugs are antagonists of the dopamine receptor on mammotrophs[24]; the potency of some of these compounds has been estimated *in vitro*[30]. In addition, antipsychotic drugs are powerful antagonists of the presynaptic dopamine receptors of the rabbit sympathetic neurones[48] and the autoreceptors on the dopamine-containing nigro-neostriatal neurones[49]. Antipsychotic drugs are also antagonists of the dopamine receptor which is linked to striatal adenylyl cyclase[6,11,18,19].

Among the different classes of antipsychotic compounds, only the butyrophenone series contributes to the identification of distinct types of receptors for dopamine. Butyrophenones, such as haloperidol, are less potent antagonists of the dopamine receptor linked to adenylyl cyclase than would be anticipated from their potency as antipsychotic agents[57] or antagonists of the dopamine receptor in the anterior pituitary[30]. Other antipsychotic compounds do not display such differential activity. For example, the dopamine receptors in the anterior pituitary and those linked to striatal adenylyl cyclase display approximately equivalent affinities for members of the thioxanthine series (for example, *cis*-fluphenthixol), the thiothixene series (for example, *cis*-thiothixene) and (+)butaclamol[6,11,18,19,30].

Radiolabelled ligands

A number of dopaminergic drugs, radiolabelled to high specific activity, havesx used to identify specific neuronal binding sites which may correspond to dopamine receptors. Impressive correlations have been established between the affinity of certain dopaminergic drugs for these specific binding sites and their potency in animal and human tests of central dopaminergic neurotransmission[58]. The physiological and biochemical function of the central dopamine receptors identified by binding studies are only just beginnimv be understood.

The dopamine receptors in the anterior pituitary have been studied with three different radiolabelled ligands, spiroperidol[32], dihydroergocryptine[30] and dopamine[31]. Because the properties of this receptor are well characterised, a correlation between the binding sites and the receptor has been possible[30,31]. There is no direct evidence to suggest that any of the binding sites identified in the striatum with radiolabelled dopamine[58], haloperidol[4,59], spiroperidol[32], apomorphine[60], dihydroergocryptine[61] or ADTN[62] are directly related to the dopamine receptor which regulates adenylyl cyclase activity in this region. Recent studies with kainic acid lesions and ablations of the cerebral cortex provide further evidence to distinguish some of the specific binding sites from the dopamine-sensitive adenylyl cyclase in the striatum[63]. These experiments imply that the striatal dopamine-sensitive adenylyl cyclase is located predominantly on neurones intrinsic to the caudate; in contrast, most of the striatal haloperidol binding sites are located on nerve terminals derived from neurones in the cerebral cortex. Recently, specific binding sites with properties similar to the dopamine receptor regulating striatal adenylyl cyclase have been identified in experiments with radiolabelled *cis*-flupenthixol[64].

A classification of dopamine receptors

It is evident that dopamine receptors can be divided by biochemical or pharmacological criteria. The bovine parathyroid provides an example of a type of dopamine receptor which stimulates the enzyme adenylyl cyclase, whereas the mammotrophs of the anterior pituitary contain dopamine receptors which do not stimulate adenylyl cyclase. We propose that the dopamine receptors in these two tissues are examples of two distinct categories of receptor. The properties of these two types of dopamine receptors, which we designate D-1 and D-2, are summarised in Table 1. This is only the first level of division in the classification of dopamine receptors; it is likely that with further examination, particularly as pharmacological evidence accumulates, subcategories of each type of receptor will be discerned. For example, the autoreceptors on terminals of the nigro-neostriatal neurones can be differentiated from the dopamine receptors in the anterior pituitary[66].

Discussion

As an example of the location of different dopamine receptors within a tissue, it is of interest to examine the striatum, the most extensively studied dopaminergic brain region. We are able to identify dopamine receptors at five sites within the nigro-striatal axis (Fig. 1). The dopamine receptors at three of these sites are not associated with adenylyl cyclase (and therefore would be designated D-2 dopamine receptors in our classification); at two of the sites, the dopamine receptors are linked to adenylyl cyclase (and therefore would be designated D-1 dopamine receptors). In the striatum there are: (1) presynaptic receptors which control tyrosine hydroxylase and are unassociated with an adenylyl cyclase; (2) postsynaptic receptors responding to ergot agonists *in vivo*, also unrelated to an adenylyl cyclase; and (3) postsynaptic receptors closely associated with an adenylyl cyclase. Furthermore, in the substantia nigra there are: (4) presynaptic dopamine receptors (which are associated with adenylyl cyclase); and (5) autoreceptors (unassociated with adenylyl cyclase). From current knowledge, it is not possible to construct a theoretical framework ascribing a function, in terms of motor control, to any of these various dopaminergic mechanisms.

The concept of multiple categories of dopaminergic receptors has important implications for therapeutic medicine. Stimulation of D-1 receptors increases renal blood flow[50]. Dopaminergic agonists are currently used to treat circulatory shock; the pressor agents previously used had the disadvantage of producing renal vasoconstriction and thus decreasing the already compromised vascular perfusion of the kidneys.

Drugs which modify activity at the D-2 receptor have been exploited to treat endocrinological, neurological and psychiatric disorders. Bromocriptine, an agonist for the D-2 receptor, is the most satisfactory drug for stopping lactation in normal post-partum women or for pathological lactation in both women and men[25,67]. Up to 30% of women with secondary amenorrhoea and infertility have hyperprolactinaemia; in the majority of such patients, serum prolactin is reduced by bromocriptine, with consequent restoration of menstruation and fertility[68]. Bromocriptine has also been reported to decrease premenstrual tension in normal women[69] and to improve potency in men with hyperprolactinaemia[68]. In acromegalic subjects dopaminergic agents decrease the pathologically elevated levels of growth hormone; bromocriptine constitutes the first medical form of treatment for acromegaly, reversing the clinical consequences of abnormally high growth hormone concentrations[70]. The neurological features of parkinsonism seem to result from inadequate transmission at certain D-2 receptors. Bromocriptine has a therapeutic effect in the disease[71], and the selective D-2 antagonist, molindone, induces the syndrome[72]. Dopaminergic blocking drugs are the cornerstone of treatment of schizophrenia; most of the agents involved are antagonists of both D-1 and D-2 receptors, but molindone, is an effective agent for treating schizophrenia[72]. In support of D-2 receptor involvement in schizophrenia, it is notable that bromocriptine can induce a florid paranoid psychosis[71].

These examples suffice to indicate that if different categories of receptors mediate the various clinical results of manipulating dopaminergic activity, better treatment should be attainable by developing more specific drugs. At present there are no selective drugs for stimulating or inhibiting D-1 receptors in man, but recent animal studies suggest that such agents will be developed[73,74]. The desire to identify selective dopaminergic agonists and antagonists derives from the therapeutic successes which have been gained in the pursuit of such compounds for adrenoreceptors, histamine receptors, and acetylcholine receptors.

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articles

Towards a theory for the uranian rings

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Interparticle collisions, radiation drag and differential precession all tend to disrupt the rings of Uranus. The first two effects lead to radial spreading which would disrupt a free ring in $\lesssim 10^8$ *yr. We propose that the rings are confined in radius by gravitational torques from a series of small satellites that orbit within the ring system. Differential precession tends to destroy the apse alignment of the elliptical* ε *ring. We suggest that apse alignment is maintained by the self-gravity of the ring. The resulting mass of the* ε *ring is* $\sim 5\times10^{18}$ *g. Its radial confinement requires (for example) a pair of satellites of mass* $\sim 10^{19}$ *g, in circular orbits roughly 500 km away on either side of the ring.*

OCCULTATION observations have revealed that Uranus is surrounded by at least nine narrow rings[1-4]. The correct understanding of the origin and evolution of the rings has proved elusive. An early attempt to associate the five original rings with a series of orbital resonances involving known satellites was unsuccessful[5-7]. The subsequent discovery of four additional rings has made this approach less attractive.

The survival of narrow rings poses difficult dynamical problems.

Dynamical problems

Unconstrained rings tend to spread due to particle collisions. The characteristic diffusion time t_D is comparable to the time a particle requires to random walk across the ring[8,9],

$$t_D \sim t_c(\Delta r/\delta r)^2 \sim (\Delta r)^2/\nu \qquad (1)$$

where t_c is the collision time, ν is the kinematic viscosity, Δr is the radial width of the ring and δr is the typical semi-major axis difference of colliding particles. Because the optical depth is at least of order unity, $\Omega t_c \lesssim 1$, where Ω is the orbital angular velocity. The minimum value for δr is the mean particle radius d, so that $\nu \gtrsim d^2\Omega$. With parameters appropriate for Uranus' rings $t_D \lesssim 2\times10^8\ (\mathrm{cm}/d)^2\ (\Delta r/10\ \mathrm{km})^2$ yr.

The Poynting–Robertson effect limits the lifetime of a ring composed of small particles. The torque on an optically thick ring of radius r due to radiation drag is

$$T_{PR} \sim -\frac{L_\odot \Omega r^3 \Delta r}{c^2 a_\delta^2} \qquad (2)$$

where $L_\odot$ is the solar luminosity and a_δ is Uranus' semi-major axis. The orbital decay time for a ring of surface density σ is

$$t_{PR} \sim 4\times10^9(\sigma/\mathrm{g\ cm^{-2}})\ \ \mathrm{yr} \qquad (3)$$

For the two resolved rings (β and ε) the optical depth $\tau \sim 1$ (refs 3, 4). Because $\sigma \sim \rho d\tau$, where the particles are composed of material of density ρ, the decay time is

$$t_{PR} \sim 4\times10^9(\rho/\mathrm{g\ cm^{-3}})(d/\mathrm{cm})\ \ \mathrm{yr} \qquad (4)$$

A ring with a range of particle sizes would spread in the much shorter time

$$t'_{PR} \sim t_{PR}(\Delta r/r) \sim 10^6(\rho/\mathrm{g\ cm^{-3}})(d/\mathrm{cm})(\Delta r/10\ \mathrm{km})\ \ \mathrm{yr} \qquad (5)$$

where $r \sim 5\times10^4$ km.

Combining the relationships for t_D and t'_{PR}, we find that the maximum survival time for a free ring is of order 6×10^6 $(\rho/\mathrm{g\ cm^{-3}})^{2/3}(\Delta r/10\ \mathrm{km})^{4/3}$ yr, and is obtained for a ring composed of particles with masses $\sim(\Delta r/10\ \mathrm{km})$ kg. Therefore, if the rings are not very young, they must be confined. Three lines of evidence point to confinement. First, if the rings are young and unconfined, there should also be older, more diffuse rings visible in the occultation data, and these are not detected. Second, there is very little inter-ring material. A direct map of the rings at $\lambda = 2.2\ \mu$m indicates that the total light reflected by inter-ring material is less than that reflected by the rings, implying a mean $\tau \lesssim 10^{-3}$ between the rings[10]. Third, the ε ring has very sharp edges, which implies that it is not spreading.

Associated satellites

It is plausible that the rings are associated with small, as yet undiscovered, satellites. A satellite can resist tidal disruption at the location of the rings if it is either fairly dense, $\rho_s \gtrsim 2\ \mathrm{g\ cm^{-3}}$, or small, $R_s \lesssim 100\ \mathrm{km}\ \ (p_m/10^7\ \mathrm{dyn\ cm^{-2}})^{1/2}\ \ (\rho_s/\mathrm{g\ cm^{-3}})^{-1/2}$, where p_m is tensile strength ($p_m \sim 10^7$ dyn cm^{-2} for ice and $\sim 10^9$ dyn cm^{-2} for silicates or metals)[11].

For the moment assume that both the satellite orbits and the rings are circular. Consider a satellite separated from the ring by a distance x. The satellite exerts a gravitational torque T_s which tends to repel the ring:

$$T_s \sim \pm f_1 \frac{G^2 m_s^2 r\sigma\Delta r}{\Omega^2 x^4} \qquad (6)$$

where m_s is the satellite mass, Δr is the ring width, σ is assumed to be uniform across the ring, and f_1 is of order unity. The + and − signs apply if the satellite is inside or outside the ring respectively. Equation (6) may be obtained by a crude impulse approximation[12] or by summing the torques from all resonances of the form $\Omega = n\Omega_s/(n\pm1)$ which lie within the ring (ref. 13 and unpublished data).

It is valid for $|\Delta r| \ll |x| \ll r$. Diffuse material between two satellites will be driven into a ring on which the external torque is zero. The external torque deposits angular momentum in the inner part of the ring and removes it from the outer part. Angular momentum is transported outwards by shear stresses due to differential rotation and particle collisions. The ring will narrow until these two processes balance. In equilibrium

$$f_1\frac{G^2 m_s^2 r\sigma\Delta r^2}{\Omega^2 x^5} \sim 2\pi r^3\sigma\nu\left|\frac{d\Omega}{dr}\right| \qquad (7)$$

In equation (7) we assume that both satellites have the same mass. The left hand side is the magnitude of the net external torque on either half of the ring and the right hand side is the

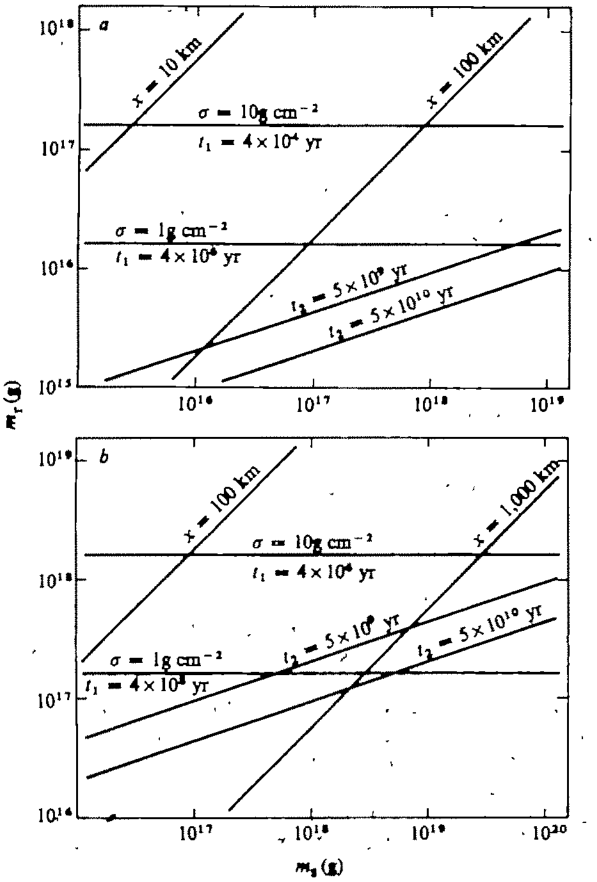

Fig. 1 Lines of constant satellite distance x, surface density σ, formation time t_1 and disruption time t_2 for rings of width: a, 5 km; and b, 50 km. If the satellites are trapped by resonances the value of t_2 is irrelevant. The lines are plotted for $f_1 = f_2 = 1$ and particle density $\rho = 1$ g cm^{-3}. For fixed Δr, m_r and m_s, $x \propto \rho^{2/5}(f_1/f_2)^{1/5}$, $t_1 \propto \rho^2 f_2^{-1}$, $t_2 \propto \rho^2 f_2^{-1}$.

magnitude of the torque associated with the shear stress. As discussed earlier $\nu \gg d^2\,\Omega$.

We now set $\nu \sim f_2\Omega(\sigma/\rho)^2$. For a monolayer of particles with optical depth of order unity, $f_2 \sim 1$. We neglect the Poynting–Robertson torque, which is as important as the torque due to shear stresses for $d \lesssim 10$ cm. We argue below that the average particle size in the ε ring exceeds this limit.

Before viscous stresses become important, the ring narrows at rate $\mathrm{d}(\Delta r)/\mathrm{d}t = \Delta r/t_1$, where

$$t_1 \sim \frac{\Omega^3 r x^5}{f_1 G^2 m_s^2} \tag{8}$$

Using equation (7) it is easy to see that t_1 is just the diffusion time t_D (see equation (1)) for the ring in its equilibrium state. This time is less than the age of the Solar System for a particle size $d \gg 1$ cm.

If the satellites are unconstrained they recede from the ring. The characteristic time in which a satellite doubles its distance from the ring is

$$t_2 \sim (4m_s/m_r)t_1 \tag{9}$$

where m_r is the ring mass,

$$m_r = 2\pi r\sigma\Delta r \tag{10}$$

Sample solutions of equations (7)–(10) are shown in Fig. 1a and b. Not all regions of these diagrams correspond to allowed solutions. Our picture is only valid if several resonances from each satellite lie within the ring. This requires $x \lesssim (r\Delta r)^{1/2} \sim 700\,\mathrm{km}\,(\Delta r/10\,\mathrm{km})^{1/2}$. A similar limit is suggested by the requirement that there is at least one satellite between every two rings, as there are at least nine rings in an interval of 10^4 km. If the satellites are not constrained, we must have $t_2 \lesssim 5\times10^9$ yr. However, the satellites may be trapped in resonances with the known satellites of Uranus. For the corotation resonances discussed previously[5–7] the satellites can be held in resonance if the dimensionless strength of the resonance satisfies

$$\mathcal{R} \gg f_1 \frac{G^2 m_s m_r}{r^2\Omega^4 x^4} \tag{11}$$

Here $\mathcal{R}$ is the amplitude of the resonance term in the disturbing potential in units of $GM_{♅}/a$. In general there are a number of resonances between the rings for which inequality (11) is satisfied[7].

Precessing ellipses

Another remarkable feature is that some of the rings are precessing ellipses. In particular, the inner and outer edges of the ε ring can be fitted by aligned keplerian ellipses with semi-major axes $a \pm \Delta a/2$ and eccentricities $e \pm \Delta e/2$, where $a = 51{,}284$ km, $e = 7.8\times10^{-3}$, $\Delta a = 60$ km and $\Delta e = 7.2\times10^{-4}$. The α, β and 4 rings also seem to be elliptical. The precession is presumably due to the quadrupole moment of Uranus. The observed rates for the α and ε rings together with the formula

$$(\mathrm{d}\tilde{\omega}/\mathrm{d}t)_Q = \frac{3}{2}J_2 n\left(\frac{R_{♅}}{a}\right)^2 \tag{12}$$

yield $J_2 = 3.43\times10^{-3}$ for $R_{♅} = 26{,}200$ km (ref. 4). Naive application of equation (12) predicts that the inner edge of the ring would precess by one revolution relative to the outer edge in 200 yr. Thus the observed alignment of the inner and outer edges must be explained.

The simplest explanation is that the apsides of the ring particles are locked together by the self-gravity of the ring. Consider two particles of mass m moving on aligned elliptical coplanar orbits, with orbital elements ($a \pm \delta a/2$, $e \pm \delta e/2$, where $0 < \delta a/a \ll 1$, $|\delta e|/e \ll 1$, $e \ll 1$. It can be shown that as a result of their mutual gravitational interaction there is a contribution to the precession rate

$$\left(\frac{\mathrm{d}\tilde{\omega}}{\mathrm{d}t}\right)_G = \pm\frac{na}{\pi}\frac{m}{M_{♅}}\left[(\delta a^2 - b^2)^{-1/2}\left(\frac{\delta a}{eb} - \frac{1}{2}\right) - \frac{1}{eb}\right] \tag{13}$$

Here $b = a\delta e + e\delta a$ and we have assumed that the orbits do not cross, so that the quantity inside the square root is positive.

We can determine the mass m_r of the ε ring by dividing it into a large number of subrings, applying equations (12) and (13) to calculate the precession rate of each subring, and requiring that all these rates are the same. A crude estimate of m_r is obtained by making two severe approximations: (1) only two subrings are used; (2), it is assumed that $|b/\delta a| \ll 1$. Then we find

$$\frac{m_r}{M_{♅}} = \frac{21\pi}{2}\frac{e}{\delta e}\left(\frac{\delta a}{a}\right)^3 J_2\left(\frac{R_{♅}}{a}\right)^2 \tag{14}$$

Identifying δa and δe with half the total variation in semi-major axis and eccentricity across the ε ring yields $m_r = 1.1\times10^{19}$ g. A more careful calculation using many subrings and the exact form of equation (13) yields $m_r = 4.8\times10^{18}$ g, corresponding to a mean surface density $\sigma = 25$ g cm^{-2}. We have investigated the precession of rings with a wide variety of surface density profiles and find that m_r is insensitive to the details of the profile. Moreover, the eccentricity is always larger at the outer boundary than at the inner boundary, in accord with observations of the ε ring.

We have considered three other potential mechanisms for maintaining apse alignment in a particle ring. First, the satellites which confine the ring may induce differential precession which cancels that due to the planet's quadrupole moment. However, application of equation (13) shows that the satellite's effect is negligible. Second, interparticle collisions give rise to an effective hydrodynamic pressure which modifies the particle orbits. It is easy to show that smooth pressure gradients are inadequate to overcome the differential precession. Third, a discontinuity analogous to a hydrodynamic shock may form in the ring. A preliminary investigation suggests that, although the induced precession could be significant, it would not maintain rigid precession.

Unanswered questions

Even if the model presented here is correct, there remain many unanswered questions. We have not discussed the origin of the ring material. The approximate equality of the masses of the rings and their confining satellites suggest that the rings may result from the collisional breakup of the satellites. A more speculative possibility is that the rings may reform into satellites.

Our confinement theory is strictly applicable only for circular rings and satellite orbits. Furthermore, we have not investigated the growth and decay of the eccentricities of the rings. It seems plausible that the satellite torques which confine a ring also tend to increase its eccentricity. On the other hand, dissipation due to shear stresses tends to decrease the eccentricity.

If we introduce the surface density deduced for the ε ring into the equations for the confinement of circular rings (see Fig. 1*b*), we find that t_2 is much less than the age of the Solar System. There are several possible explanations. The uncertainty in ρ, f_2 and x means that $t_2 > 5 \times 10^9$ yr cannot be ruled out. Also, the confining satellites may be trapped in resonances. A confinement theory for elliptical rings might yield much larger values of t_2. Finally, the time required for rings to form into satellites may be much less than the age of the Solar System.

The processes which determine the particle size distribution and optical depth in planetary rings are not understood. If there is a substantial population of small particles, the analysis must be modified to include the Poynting–Robertson effect.

The sharp edges of the ε ring pose a challenge to any theory. They suggest that a portion of the constraining torques must be applied very near the ring boundaries.

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UV spectrum of supernova remnant reveals carbon depletion in the interstellar medium

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The first observation of the far UV, emission line spectrum of a supernova remnant (SNR), namely a filament in the Cygnus Loop, suggests that carbon, initially depleted in the gaseous phase by a factor of 10, is progressively returned to this phase, presumably by destruction of graphite grains in the high temperature, post-shock region.

MODELS for the cooling and recombination of a collisionally ionised gas[1,2] such as is found in SNRs indicate that UV resonance lines of various elements dominate the emission spectrum in the UV–near IR region. In particular carbon, a key element in nucleosynthesis, has no prominent optical lines but emits strong UV lines in different stages of ionisation which can be used to determine accurate abundances.

Previous UV experiments have not looked at SNRs because of the extended nature and faintness of the filaments. We discuss here the first UV spectrum of a SNR obtained with the IUE satellite launched in January 1978 as a combined effort by NASA, ESA and the SRC. The IUE instrument and its performance are described elsewhere[3].

One of the brightest filament of the Cygnus Loop, known as position 1 in a previous study in the optical region[4], was selected for the observation. Filaments such as this are explained as being due to radiative shocks of velocity 90–120 km s^{-1} moving into a medium of density ~6 cm^{-3} (refs 2, 4, 5). This is consistent with the filamentary expansion velocity (~120 km s^{-1}), but is much lower than that inferred from X-ray data. All observations[6-8] favour a plasma temperature of 2–4 × 10^6 K which would imply a shock velocity of about 400 km s^{-1}. The discrepancy has been explained as being due to denser cloudlets embedded in lower density material[10].

It is thought that the UV lines arise from shocks driving into these higher density cloudlets. Although no information can be obtained on the pre-shock density, the UV data can be used to derive a very accurate shock velocity, as described below.

UV spectrum of a filament of the Cygnus Loop

A single, three-hour exposure was obtained in April 1978 in the 1,200–2,000 Å wavelength region using a blind offset procedure. Several emission lines are present including resonance transitions of C, N, O and Si plus the He II 1,640 Å emission. The strength of the geocoronal Lyα prevents any reliable measure of the Lyα emission from the Cygnus Loop. A continuum emission is detected along the full spectrum. The standard data reduction technique[3] was applied. At the time of writing an official absolute calibration for the IUE spectrographs was not available: to derive the relative intensities of the emission lines we have adopted a provisional calibration obtained by

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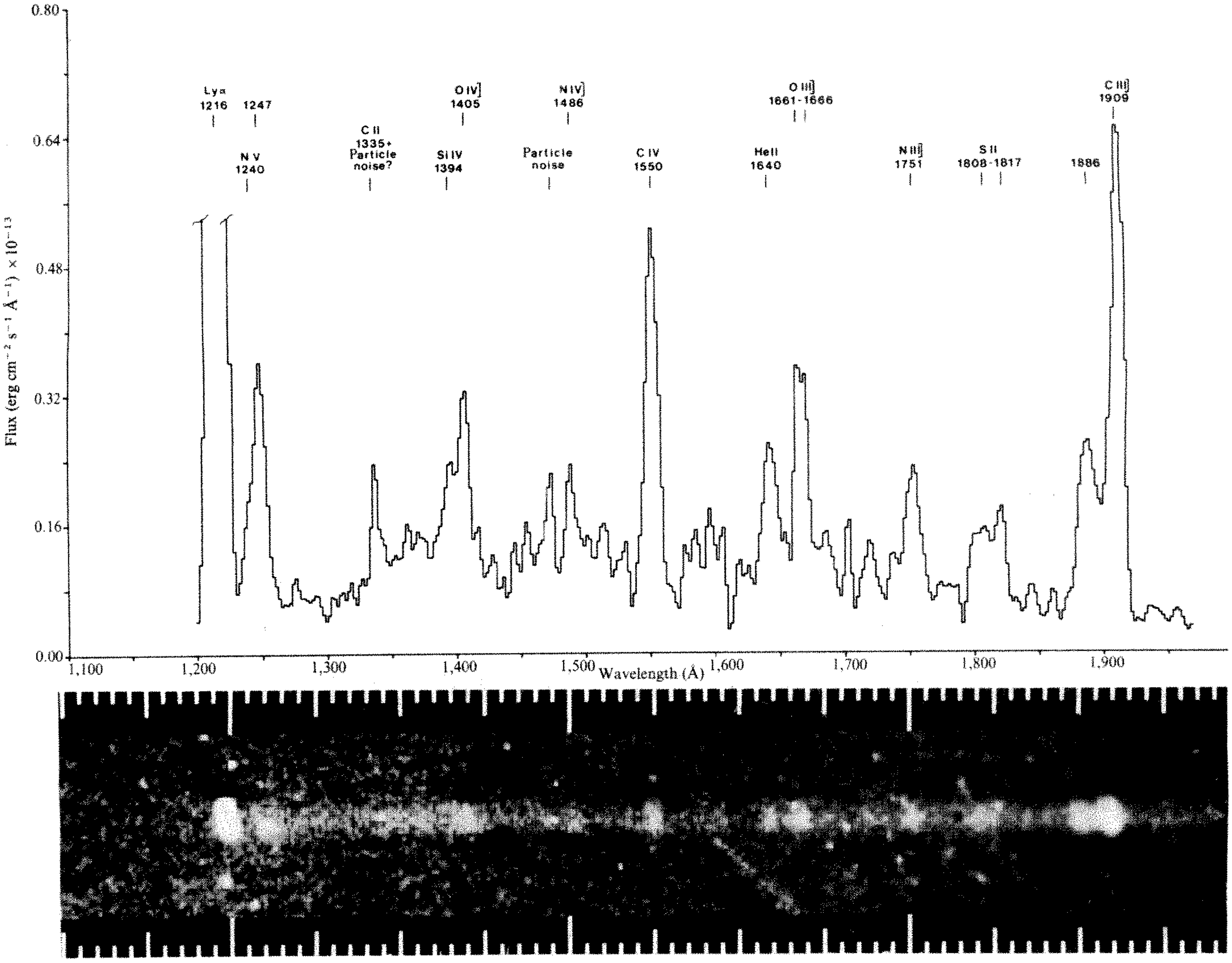

Fig. 1 The UV spectrum of a filament in the Cygnus Loop in the 1,200–2,000 Å range and the resulting intensity plot with the identified emission lines. Several particle events appear as bright points in the three hours exposure.

R. Bohlin from spectra of the star μ Col. As the errors are still uncertain, we will discuss the continuum distribution and the absolute line intensities elsewhere.

Figure 1 shows the reduced spectrum and the resulting intensity plot with the identifications of the emission lines. The spectrum has been smoothed and it is not corrected for interstellar absorption. The feature at 1,393 Å is probably a blend of Si IV 1,394 Å and S IV 1,385 Å, although the silicon should always be about four times stronger than sulphur. Two features at 1,247 Å and 1,886 Å are still unidentified: both seem too far from the plausible lines of Si II at 1,260 Å and Si III at 1,892 Å. Note that a small feature assigned to N V at 1,240 Å is really present even if it has disappeared from the smoothed tracing; its intensity has been measured from the original spectrum. The unidentified feature at 1,247 Å may be a diffuse particle event. Intensities relative to the C III 1,909 Å line are given in Table 1. The estimated error, due to uncertainty in the sensitivity curve and in some steps of the data extraction, is ~30%.

Physical conditions in the filament

The UV data can be used to derive a very accurate shock velocity. The reason for this is evident from consideration of shock models[1,2,5,11]. In the immediate post-shock gas, collisional ionisation increases the state of ionisation of the incoming gas, but, at the same time, cooling processes rapidly reduce the temperature so that the gas never has time to reach collisional ionisation equilibrium, although the maximum state of ionisation will follow the same sort of temperature dependence. Provided that hydrogen and helium are pre-ionised (which occurs[11] for shock velocities >110 km s^{-1}), this maximum state of ionisation is just a function of the post-shock temperature.

As the gas cools rapidly, the temperature at which recombination occurs is effectively dependent only on atomic parameters, and is therefore independent of shock parameters. For species of very low ionisation potential such as C I and S I, the effects of photoionisation by the UV photons generated in the hot plasma become important and this conclusion is no longer valid.

With this simple model a semi-empirical function can be constructed for the ratio of two UV lines produced by ionic species, A^{+n} and $A^{+(n+1)}$, of the same atom. The ionisation balance between the ionic species will be of the form:

$$\frac{N(n+1)}{N(n)} = C_1 T^m \exp\left(-\frac{I_n}{KT}\right) \quad (1)$$

Where I_n is the ionisation potential of ion A^{+n} whose number density is $N(n)$. The power m depends on the recombination coefficient, but should be ~1.3 for a wide range of atoms. The flux produced in UV lines is given by

$$F(n) = C_2 T^{-1/2} \bar{G}(T, Z) \exp(-E_n/KT) N(n) N_e \quad (2)$$

Where E_n is the excitation potential of the transition considered, and $\bar{G}(T, Z)$ is a Gaunt factor of the order unity, a function of both temperature and atomic charge Z (refs 12, 13). From equations (1) and (2) a fairly good approximation for the ratio of the two lines is:

$$\frac{F(n+1)}{F(n)} = C_3 T^m \exp\left(-\frac{I_n + E_{n+1} - E_n}{KT}\right) \qquad (3)$$

In practice, the temperature of the zones producing the two lines will be somewhat different, but this will be ignored in the spirit of the approximation. Hence:

$$\ln\frac{F(n+1)}{F(n)} = \ln C_3 + m \ln T - \frac{I_n + E_{n+1} - E_n}{KT} \qquad (4)$$

At low temperature, the last term on the right-hand side dominates, and T can be given by the post-shock temperature. In terms of shock velocity:

$$\left(\frac{150{,}000\ \mathrm{K}}{T}\right) = \left(\frac{100\ \mathrm{km\ s^{-1}}}{V}\right)^2 \qquad (5)$$

from shock models. Hence, expressing velocities in units of 100 km s^{-1} and ionisation and excitation potentials in electron volts, at low shock velocity, the dimensionless function;

$$\log\frac{F_{n+1}}{F_n} = C_4 - \frac{I_n + E_{n+1} - E_n}{29.75\ \mathrm{eV}}\left(\frac{100\ \mathrm{km\ s^{-1}}}{V}\right)^2 \qquad (6)$$

will correspond to a straight line of slope -1.

On the other hand, at high shock velocity the ratio of the two lines will tend to be constant because they are produced in two zones whose temperatures and thicknesses are in constant ratio. The graph should therefore become a horizontal straight line at high shock velocities.

At intermediate velocities, two effects working in opposite directions have to be taken into account. First, the mean temperature of the zone emitting the $(n+1)$ species line does not increase as rapidly as in equation (5) when this zone becomes thick, but only half as rapidly. This would tend to produce a line of slope -0.5, but for the effect of the second term on the right of equation (4). For $m = 1.3$ this tends to steepen the slope by -0.169, so at intermediate temperature, the curve should have a slope -0.669 or about $-2/3$.

Figure 2 shows the universal curve so obtained for all temperature-sensitive ratios which reach saturation at the highest velocities[2]. Using this in conjunction with the shock models referred to above we derive the shock velocities for the Cygnus Loop observation given in Table 1. The carbon ratios give somewhat lower values than the rest; these are not used for reasons given below. We conclude that, for the region observed, the probable shock velocity is 121 ± 10 km s^{-1}, in excellent agreement with the optical values.

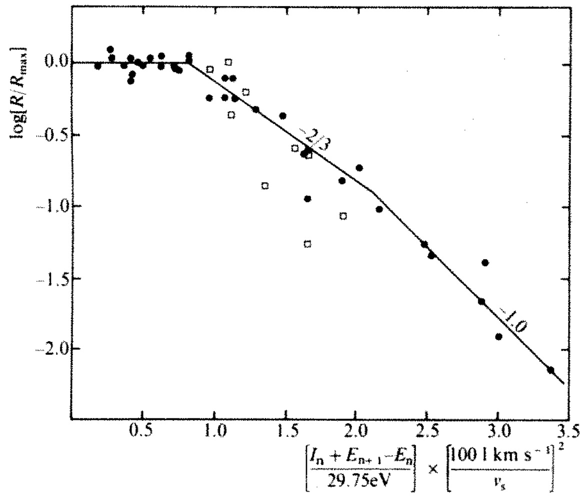

Fig. 2 A plot of theoretical line intensity ratios versus a function of atomic constants and shock velocity. The ratios R are expressed in units of $R_{\max}$, the constant value which is reached at high velocities. I_n is the ionisation potential and E_n the excitation potential for the considered ion and transition respectively. ●, Represent ratios from models which assume full pre-ionisation over the whole velocity range[2], whilst squares are from models with incomplete pre-ionisation.

Table 1 Shock velocities for Cygnus Loop observations

Ratio	Shock velocity (km s^{-1})
O IV λ1406/O III λ1663	117–135
N V λ1240/N III λ1750	126–130
N IV λ1485/N III λ1750	102–118
C IV λ1549/C III λ1909	93–99
C III λ1909/C II λ1335	>88

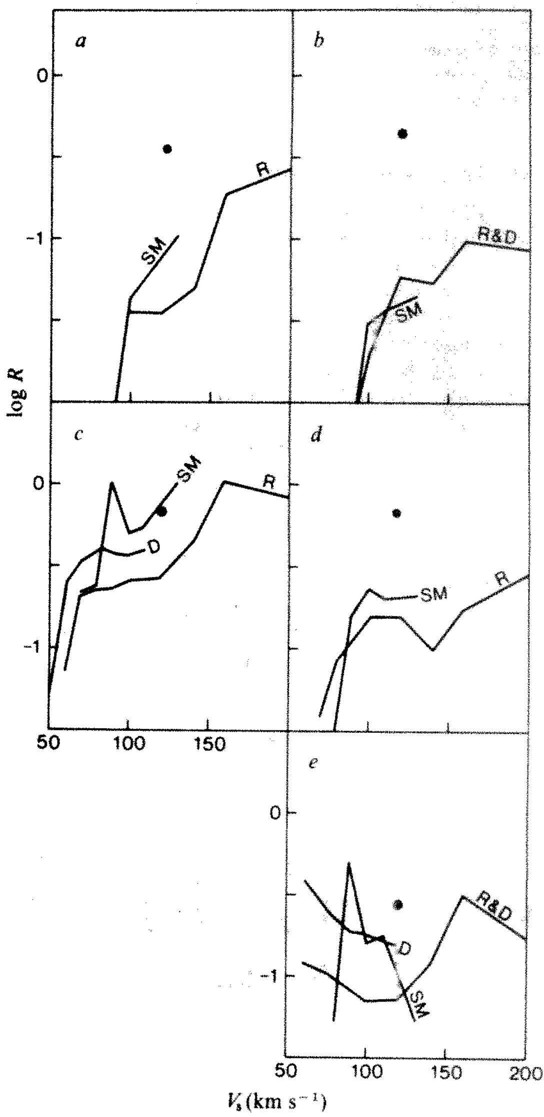

Fig. 3 The lines give the trend of various line intensity ratios as a function of shock velocity for different models (Raymond[2](R), Shull and McKee[11](SM), and Dopita[1](D)) assuming standard abundances. The position of the Cygnus Loop observation (●) suggests an underabundance of all ions of carbon with respect to O, N and Si+S. *a*, O IV/C IV = 1,406/1,549; *b*, N IV/C IV = 1,485/1,549; *c*, O III/C III = 1,663/1,909; *d*, (Si IV + S IV)/C III = (1,394 + 1,385)/1,909; *e*, N III/C III = 1,750/1,909.

Table 2 UV emission line intensities

Line	λ (Å)	Observed	Computed from model X	F	(F+G)	F_d	$(F+G)_d$
C III]	1,909	100	100	100	100	100	100
N III]	1,750	30	33*	8*	12	20*	30
O III]	1,663	68	128	28	83	70	207
He II	1,640	36	—	—	36	—	90
C IV	1,549	80	165	252	350	63	88
N IV	1,485	34	13	14*	14	35*	35
O IV]	1,405	27	23	9	30	22	75
Si IV S IV]	1,393	36	2†	16†	22	40†	55
C II	1,335	48	34	42	51	66	81
N V	1,240	31	2	2	14	5	35

X is a carbon-depleted model with $v_s = 100\ \mathrm{km\ s^{-1}}$; F has no depletion and $v_s = 110\ \mathrm{km\ s^{-1}}$; both are from Raymond's work[2].

(F+G) is an average from two undepleted models of Shull and McKee[11]. F_d and $(F+G)_d$ are synthesised models with carbon depleted in ratios.

1.0; 0.4; 0 2 in the logarithm for C IV, C III, and C II respectively.

* Not given in the original computation. Estimated from calculated intensities of the same species with the help of Dopita[1] models.

† S IV not given in Raymond computation. Estimated from Shull and McKee[11] models.

Determination of chemical abundances

As no hydrogen lines are seen, and the spectrum is dominated by the lines of carbon, we derive the abundance of ionic species with respect to the carbon species of closest ionisation potential. This ensures that the error made in mismeasurement of shock velocity is relatively unimportant.

The Cygnus Loop observation, when compared with the results of various shock models, reveals that all other elements (O, N and Si+S) are overabundant with respect to carbon. For the comparison with C III, this effect seems to be $+0.4 \pm 0.2$ in the log, and by comparison with C IV the effect is larger, amounting to $+1 \pm 0.15$ in the log. This effect can either be treated as an underabundance of carbon, or an overabundance of everything else. However, the former is the simplest hypothesis, and we adopt this here. In any event, observations of interstellar dark clouds[14] lead to underabundances of carbon in the gaseous phase of about a factor 10, very similar to the C IV value derived here which is ascribed to graphite grain formation.

In a radiating shock, such graphite grains would pass into the high-temperature zone, and if they are destroyed by the passage through the radiating zone, this would show up as a progressive repletion of carbon in the gaseous phase towards lower temperature. This effect is precisely what is observed here, C IV being 0.6 (in the log) less abundant than C III. At risk of overinterpreting the data, the C II/C III λ1335/λ1909 ratio the data suggests a depletion of 0.2 ± 0.2 for C II.

Introducing depletions of 1.0:0.4:0.2 (C IV:C III:C II) makes all the data consistent. In particular, the C IV/C III λ1549/λ1909 ratio gives a low shock velocity without differential depletion. When this is included, however, the shock velocity derived from this ratio is in the range 110–123 $\mathrm{km\ s^{-1}}$, in full agreement with the other values.

This internal consistency is reflected in full shock model calculations summarised in Table 2. The Raymond model[2] X, used to reproduce the optical spectrum, had a velocity $V_s = 100\ \mathrm{km\ s^{-1}}$ with a depletion of both carbon and silicon. Although this model is a reasonable fit for many lines, it fails for the high excitation ones (such as N IV, O IV) indicating a too low velocity. Higher velocity models without depletion, F and (F+G), fail by predicting too strong C IV and other lines somewhat too weak. Only for the differentially depleted models is there a good fit, with all lines fitting the observations to within a factor of about two, which, bearing in mind the uncertainties in the atomic physics and modelling procedures, is about as good as could possibly be achieved at present.

Conclusion

There is very strong evidence that carbon, initially depleted in the gaseous phase by a factor of ~10, is progressively returned to this phase through the cooling zone, presumably by destruction of graphite grains. The mechanism of this process will be discussed in full in a later paper.

This work was based on observations by the IUE collected at the Villafranca Satellite Tracking Station of the ESA.

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Tholins: organic chemistry of interstellar grains and gas

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A complex organic solid produced from cosmically abundant molecules helps to explain many properties of the interstellar grains and gas.

FOR the past decade we have been producing[1–4] in our laboratory a variety of complex organic solids from mixtures of the cosmically abundant gases CH_4, C_2H_6, NH_3, H_2O, HCHO, and H_2S. The product, synthesised by ultraviolet (UV) light or spark discharge, is a brown, sometimes sticky residue, which has been called, because of its resistance to conventional analytical chemistry, 'intractable polymer'. However, we have recently suceeded, through sequential and non-sequential pyrolyses followed by gas chromatography–mass spectrometry (GC–MS), in determining something of the composition of this material[3,4]. It is clearly not a polymer—a repetition of the same monomeric unit—and some other term is needed. We have discussed this material as a constituent of the Earth's primitive oceans and therefore as relevant to the origin of life[1]; as a component of red aerosols in the atmospheres of the outer planets and Titan[2,5]; as

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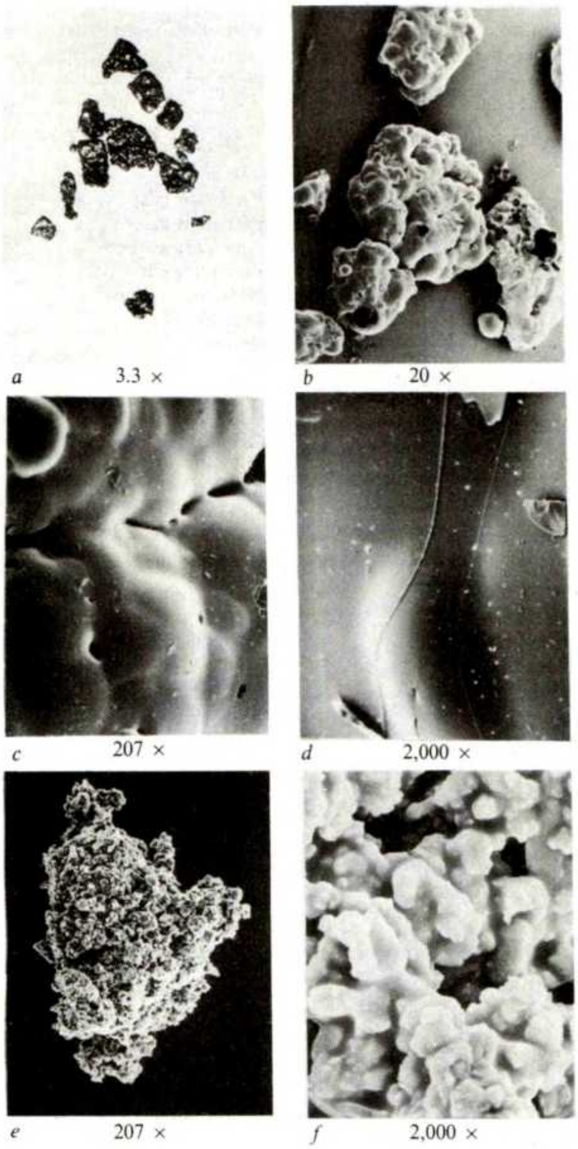

Fig. 1 Light microscopy (*a*) and scanning electron microscopy (*b*–*d*) of spark tholins heated to 920 °C. The smallest particles in (*d*) are the size of interstellar grains. Also shown (*e*–*f*) is the quite different gross structure of UV tholins without elemental S, probably rich in disulphide bridges.

present in comets, carbonaceous chondrites, and pre-planetary solar nebulae[2,5]; and as a major constituent of the interstellar medium, which is the concept discussed here. We propose, as a model-free descriptive term, 'tholins' (gk θὸλος, muddy; but also θολὸς, vault or dome), although we were tempted by the phrase 'star-tar'. The properties of tholins will depend on the energy source used and the initial abundances of precursors, but a general physical and chemical similarity among the various tholins is evident.

UV tholins are produced by near-UV photodissociation of H_2C or HCHO, and chain reactions involving the resulting superthermal H atom. Spark tholins, first made by S. L. Miller[6], are produced by electrical discharges. Both involve the high-temperature dissociation of precursor gases and subsequent low-temperature recombination of radicals. A comparison of the two tholins which we have studied most is presented in Table 1. The 50 or so identified pyrolitic fragments in each tholin extend to molecules as complex as C_3-alkylbenzenes, C_4-alkylthiophenes, C_6-alkyldisulphides, and C_5-alkylthiocyanates in UV tholins; and C_4-alkylbenzenes, C_2-alkylindanes, methylethylpyrazine, glutaronitrile, and $CH_2{=}CH{-}CH{=}CHCH_2CH_2CH_3$ (heptadiene) in spark tholins. (A full list of identified fragments can be found in refs 3 and 4.) There is evidence of amino acid precursors—perhaps polypeptides—in both tholins[4], and a variety of biologically interesting amino acids, including cystine, have been isolated on tholin hydrolysis[1]. The overall tholin structure may be a complex matrix, dotted with aromatic hydrocarbons, pyrroles, pyridines and other rings, and crosslinked with long-chain alkanes, alkenes and polypeptides, somewhat similar to the structure of kerogen or coal. Scanning electron micrographs of tholins are displayed in Fig. 1. The highest molecular weight fragments which have been identified are about 150 daltons, but fragments at 500 to 700 daltons have been observed and still heavier fragments obviously exist. The TGA thermal stability, especially for the spark tholin (Table 1), is impressive; the tholins have experienced a kind of stability natural selection during their synthesis in a UV or high energy proton environment. In their production, the UV tholins received a UV dose exceeding the interstellar near-UV energy density integrated over the lifetime of the Galaxy. The greater complexity of spark over UV tholins at highest magnifications (Fig. 1*d*, 1*f*) may be consistent with the higher stability of the former. We argue here that the tholins are a major constituent of interstellar grains and that their spallation products—similar to the pyrolytic GC–MS fragments—are the primary source of the organic molecules found by microwave line spectroscopy in the interstellar gas.

Table 1 Comparison of typical UV and spark discharge tholins

	UV[3]	Spark[4]
Initial reactants	CH_4, C_2H_6, NH_3, H_2O, H_2S (roughly equimolar)	CH_4, NH_3, H_2O ($\sim 2.5\%$)
Energy source	2,537 Å Hg lamp	High-frequency tesla coils
TGA, 50% thermal dissociation temperature	≃300 °C	≃900 °C
Atomic composition	Apart from S: H, 3.8%; C, 37%; N, 11%; O, 48%	H, 5.0%; C, 47% N, 36%; O, 12%
Principal GC–MS Pyrolytic products	Alkanes, alkenes, aromatic hydrocarbons, alkylthiophenes, alkylmercaptans, alkyldisulphides, isothiocyanates and some nitriles	Alkanes, alkenes, abundant nitriles, aromatic hydrocarbons, alkylbenzenes, indenes, indanes, pyrroles, pyridine, and pyrazines

The conventional composition assigned to interstellar grains—on the basis of their UV and IR spectra, optical frequency extinction, polarisation data and cosmic abundances—is a mixture of silicates, water ice, graphite, carborundum and iron[7–9]. The silicate and ice identifications are probably secure, on cosmic abundance grounds and also, for example, because interstellar features attributed to silicates are also seen in the circumstellar envelopes of oxygen—rich red giant stars. However, it has long been recognised that complex organic molecules provide a possible explanation for several otherwise puzzling properties of the interstellar medium. Sagan[8] argued that the *in situ* formation of the more complex interstellar gas molecules was very difficult to understand, and suggested instead that they are degradation products of the interstellar grains, which he proposed included stable complex organics, ejected from solar nebulae by radiation pressure and stellar winds in the early stages of evolution of stars later than spectral-type F. The fact that the abundances of interstellar organics do not decline steeply with heavy atom number points strongly to an origin as dissociation products of more complex organic species rather than an *in situ* synthesis from interstellar

Table 2 Proposed origin of interstellar molecules (All interstellar molecules composed of C, H, N, and O, known as of mid-1978, are listed, column numerals represent heavy atom number)

Molecule		Relevant laboratory experiment				
		Gas phase spark[30]	Interstellar frost[9]	Spark pyrolysis[4]	UV pyrolysis[3]	Other*
1. Hydrogen	H_2		X	X	X	Precursor gas
Methane	CH_4	X		X	X	Precursor gas
Methylidyne	CH, CH^+	X		X	X	Any hydrocarbon
Ammonia	NH_3	X	X	X	X	Precursor gas
Amino radical	NH_2^+	X		X	X	NH_3, any amine
Water	OH_2	X		X	X	Precursor gas
Hydroxyl	OH	X		X	X	Water
2 Ethynyl	$C\equiv CH$			X	X	Any acetyl or ethynyl group
Acetylene	$HC\equiv CH$	X	X			
Methylamine	CH_3NH_2				X^2	Tholin amino cmpds. (see text)
Methyleneimine	$CH_2=NH$			X	X	CH_3NH_2-H, $HNC+H$, etc
Cyanogen	$C\equiv N$			X	X	Any nitrile
Hydrogen cyanide & isocyanide	HCN, HNC	X	X	X	X	Many nitriles
Carbonyl	$C=O$	X		X		
Formyl	HCO, HCO^+			X	X	Any aldehyde
Formaldehyde	$HCHO$	X	X			
Methanol	CH_3OH		X			
3. Methylacetylene	$Ch_3-C\equiv CH$					Propene − H, etc.
Acetonitrile	$CH_3C\equiv N$	X	X	X	X	
Cyanamide	$NH_2C\equiv N$					Tholin amino cmpds. (see text)
Acetaldehyde	CH_3CHO	X	X	X		
Formic acid	$HCOOH$		X			
Formamide	NH_2CHO					Tholin amino cmpds. (see text)
Ketene	$CH_2=C=O$					Acetaldehyde − H, etc.
Isocyanic acid	$HN=C=O$					Formamide − H (note analogy to isothiocyanates)
Ethanol	CH_3CH_2OH		X			
Dimethylether	$(CH_3)_2O$					Methanol + CH_2
4. Cyanoethynyl	$C\equiv C-C\equiv N$					Acrylonitrile − H, Propylnitrile − H, etc.
Cyanoacetylene	$HC\equiv C-C\equiv N$					Acrylonitrile − H, etc.
Acrylonitrile	$CH_2=CHCN$			X	X	
Methylformate	CH_3COOH		X	X		
Tropionitrile	$CH_3CH_2C\equiv N$			X		
6. Cyanodiacetylene	$CH\equiv C-C\equiv C-C\equiv N$					Pentenenitrile − H, etc.
8. Cyanoctatetrayne	$CH\equiv C-C\equiv C-C\equiv C-C\equiv C-C\equiv N$					Benzonitrile ring breakage − H

(Table courtesy of L. E. Snyder.)

* All molecules cited in this column are identified either in tholin pyrolyzates or in the interstellar medium.

gas. If the presumed dissipation of the preplanetary solar nebula in our Solar System is typical of main sequence stars generally, a significant contribution[10] of organics to the interstellar medium is made, probably enough to coat silicate cores formed, for example, in circumstellar shells (see ref. 40). He suggested that the composition of the interstellar grains might be similar to that in contemporary carbonaceous chrondrites and cometary nuclei. Because interstellar grains might be abundant in preplanetary solar nebulae, and contribute to carbonaceous chondrite chemistry, we note the possibility of multiple recycling of stable organic matter between circumstellar disks and interstellar clouds.

Alternatively, we have shown experimentally[9] that near-UV irradiation at 77 K of stacked layers of HCHO, H_2O, NH_3 and C_2H_6 on a silicate matrix produced, on vapourising the frosts, a variety of simple organic molecules similar to those found by microwave spectroscopy in the interstellar medium, and suggested that organic synthesis also occurs *in situ* on ice-coated grains of silicates or other materials. For both modes of synthesis, Sagan[10] proposed that labile molecules would be preferentially destroyed, and that the steady state molecular population of the grains must, through natural selection, be exceptionally stable complex organics. Because of the Frank–Rabinowitch principle on the stability of multiply cross-linked structures, unbranched aliphatics, say, are not expected to be dominant, while polycyclic aromatics, complexly branched aliphatics and a matrix of mixed rings and aliphatics would be much more likely. Subsequently, Knacke[12] argued that complex organics, including one of our UV tholins, might help explain several unidentified or puzzling interstellar IR absorption or emission features, but was unable to secure an entirely acceptable fit.

Since this early work, very similar ideas seem to have been taken up by Hoyle and Wickramasinghe[13–17], who have paid particular attention to the growing body of data on IR absorption and emission in the interstellar medium. They showed that organic polymers display absorption in the 3 and 8 to 12 μm regions which roughly match the interstellar absorption. In fact, a very wide range of mixtures of complex organic molecules will satisfy such unrestrictive absorptivities, because the 3 μm absorption corresponds to characteristic vibrational transitions of O—H, N—H and, particularly, C—H bonds, while the 8–12 μm region is characteristic of C—C, C—O and C—N functional groups. But they then went on to attempt a more detailed match of organics with interstellar absorption, and claim to have 'identified' the formaldehyde polymer polyoxymethylene and, later, polysaccharides as major constituents of interstellar grains; and have gone still further in postulating sporopollenin, a particular category of polysaccharides found in the cell walls of spores and pollen[18], with typical empirical formulae ranging from $C_{90}H_{115}O_{10}$ to $C_{90}H_{158}O_{44}$, as a reasonable candidate for the composition of interstellar grains.

However, no plausible mechanism for producing such a polysaccharide in the interstellar medium or in circumstellar envelopes has been offered. They are not abundant products in typical experiments on prebiological organic chemistry which involve the irradiation or heating of cosmically abundant substances. They and their pyrolyzates do not appear, to the limit of experimental detectivity, in our analysis of tholins, including experiments in which H_2O is in excess. Polysaccharides are composed only of the atoms H, C and O and therefore do not reflect the high cosmic abundance of N and S, which, as the composition of tholins clearly illustrates, should be major constituents of organic molecules produced from cosmically abundant gases. Moreover, the idea that any particular molecule such as sporopollenin, or even a particular class of organic molecules such as the polysaccharides, should be typical of interstellar grains seems very unlikely; laboratory experience suggests that a very wide range of products should instead be produced under all plausible production scenarios.

There is no such thing as a common IR spectrum characteristic of all galactic sources. Absorption features are well-known in many sources near 3, 10 and 20 μm, but with significant variability from source to source (and in some cases even with time) in the band central wavelengths, depths and half widths. Additional absorption and emission features are known in some sources at 3.3, 6.0, 6.2, 6.8, 7.7, 8.6, 11.3 μm and other wavelengths. The very variability of IR spectra from source to source implies that a variety of molecules are responsible. On cosmic abundance grounds and on the basis of detailed models it seems likely that the 3.1, 9.7, and 18.5 μm features can be explained, perhaps in all cases, by water or ammonia ices and silicates. (Features attributed to ices are very rare in the general interstellar medium and are apparently found only in molecular clouds[19,20]). But even for these features many problems exist. In the source OH 26.5 + 0.6 the 3.1 μm feature is unlikely to be ice because the grain temperatures are too high although it may be due to gas phase (C_2H_2 + HCN) molecular absorption in a cool stellar atmosphere; also the grains show appreciable opacity shortwards of 7 μm where silicates are generally transparent[21]. In compact H II regions and NGC7027 there is a strong emission feature at 3.3 μm which cannot be due to ice, in turn invoked for H II regions to explain the adjacent 3.08 μm feature[22]. In OH231.8 + 4.2≡OH0739 − 14 a deep feature at 3.08 μm still has substantial wings at 2.3 μm, inconsistent with ice; in addition, the relative extinctions at characteristic wavelengths for silicates and ice are inconsistent with known oscillator strengths and cosmic abundances. Likewise the band shapes and depths of features attributed to water ice and silicates in the galactic centre[24] and in the Becklin–Neugebauer Object[13–17,25] in the Orion Nebula are inconsistent with cosmic abundances. The spectra of compact H II regions at 20 to 33 μm are not readily understood in terms of their 10 μm absorptions if silicates are invoked for both[26]. Some of the foregoing difficulties may be resolvable with tholins as additional constituents in the interstellar medium.

Figures 2–4 display the IR spectra of UV and spark tholins, and a few of their extracts and high-temperature phases, compared with a variety of other materials—including a sporopollenin; a thin layer chromatography cellulose matrix, as an example of non-sporopollenin polysaccharides; carbonaceous chondrites[27,28]; and two galactic spectra, one of which is Hoyle's 'best fit' to many galactic IR sources[29]. Spark and UV tholins exhibit substantial family resemblances in the IR; some of the differences are due to S compounds present in the latter and not the former. Heating tholins to high temperatures seems to destroy short wavelength absorption bands and increase continuum absorption. Functional groups are well-known to shift band central wavelengths by several tenths of a μm depending on physical state and adjacent chemistry. Some tholin IR absorptions may be due to collective phenomena (long polymers, for example, having different spectra from monomers); tholin spectra will vary from source to source depending on precursor abundances and energy sources at their origin, heating and irradiation during ejection from circumstellar clouds, and other effects. We believe that mixes of tholins, such as that in Fig. 3, match the galactic spectra at least as well as sporopollenin, and exhibit the additional virtue of having a plausible synthetic mechanism. The fit of four spectral features between 3 and 40 μm will occur by chance with a probability 10^{-8}. We anticipate still better agreement with interstellar spectra for mixes of tholins, ices and silicates.

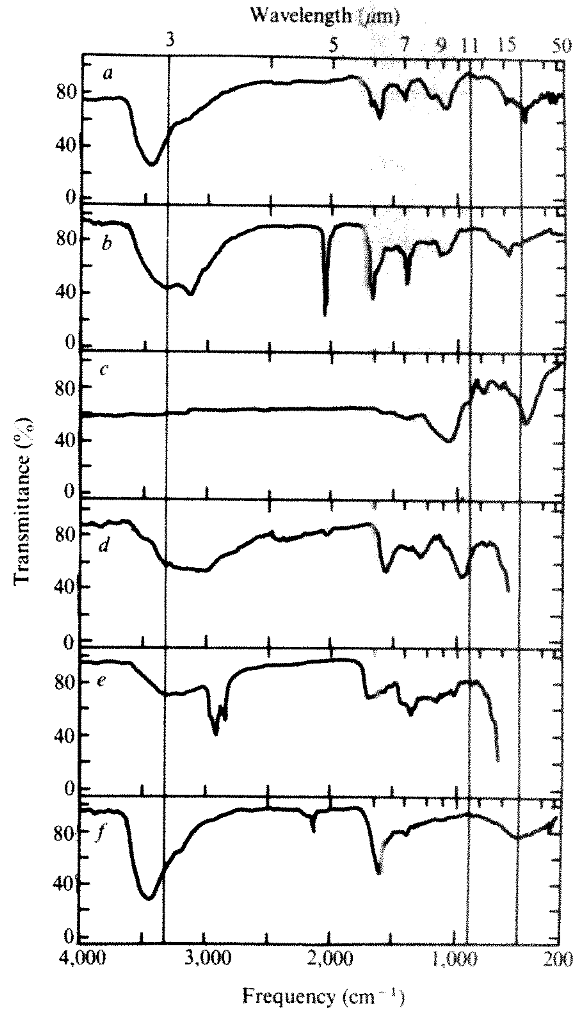

Fig. 2 Comparative IR spectra of *a*, UV tholin; *b*, UV tholin, elemental S removed; *c*, UV tholin, elemental S removed, heated to 450 °C; *d*, methanol extract of UV tholin; *e*, methanol extract of chromatographically separable yellow component of UV tholin; *f*, spark tholin. Vertical lines 3, 11 and 18 μm are for reference.

In NGC7538E, a compact IR source associated with a rich molecular cloud complex, there are unidentified features[30] at 6.0 and 6.8 μm which correlate with the presence of the 3.1 μm feature, but not the 9.7 μm feature. But in our early work on the IR spectra of UV tholins we noted[2] the presence of features at 3.42 and 6.87 μm due to long-chain alkanes, and near 6.0 μm due to alkenes. In fact the presence of these features was a major early clue that long-chain alkanes and alkenes are an important constituent of tholins, a conclusion since confirmed by GC–MS[3,4]. Willner *et al.*[30], who discovered the 6.0 and 6.8 μm features in NGC7538E, hold that a problem with the alkane and alkene identification is that the ratio of gas phase CO to grain CH_2 or CH_3 would have to be as high as 1/3; but such groups are the dominant functional groups in all analysed tholins. If the

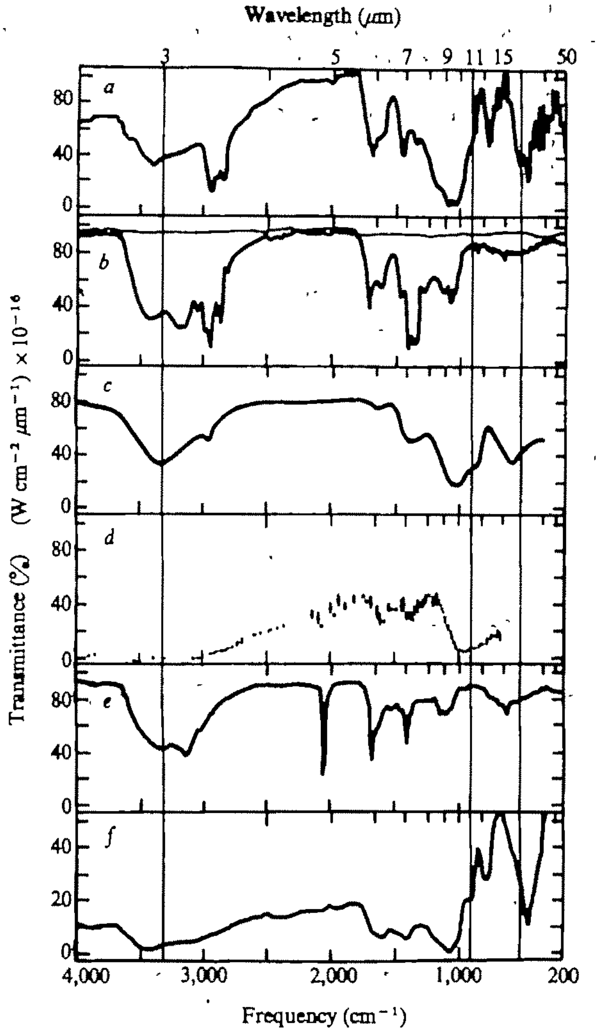

Fig. 3 Comparative IR spectra of *a*, a sporopollenin[13–17] *b*, TLC cellulose; *c*, Hoyle's[29] galactic 'best fit'; *d* NGC 7538E[30]; *e*, UV tholin – S; *f*, linear superposition of (*a*), (*c*) and (*d*) of Fig. 2. The transmission in the 760 to 200 cm^{-1} region in Fig. 2*d* is added at the 100% level.

mantles of interstellar grains are made significantly of tholins, the high methyl and methylene abundances become plausible. There is also a hint in the spectra of NGC7538E of absorption at 4.6 μm, which is characteristic of C≡C and C≡N. Features near 6.2, 7.7, 8.6 and 11.3 μm first found[31] in NGC7027—and since found[30] in many other regions of the Galaxy, as well as in M82—have analogous absorptions in several tholins (Figs 2 and 3). The presence of 8.7 and 11.3 μm features in the young IR source GL437 implies that they are not produced by anomalously abundant elements which might be present in highly evolved objects[32], but rather must be attributed to typical cosmic abundances, consistent with organic molecules.

It is a natural speculation that tholins are present in carbonaceous chondrites, and we[2] and Knacke[12] have compared relevant IR spectra. The agreement is good but not excellent for samples of Murray and Orgueil. We note here (see Fig. 4) a quite good agreement between a particular (hot-water soluble, cold-water insoluble) fraction of the Mokoia carbonaceous chondrite[27,28] and Hoyle's 'best fit' galactic spectrum[29]. However, the chemistry of Orgueil, determined[33] under identical conditions of pyrolytic GC-MS as the tholin analysis, demonstrates a relative dearth of S and N compounds (especially nitriles) in Orgueil. Depolymerisation of the Murchison carbonaceous chondrite reveals a range of organic products very similar to that of S-free tholins[33]. More work is clearly needed on the comparative organic chemistry of carbonaceous chondrites, and, in particular, of the appropriate fraction of Mokoia.

The principal UV interstellar absorption feature is at 2,200 Å, conventionally attributed to graphite, which should be formed and ejected by C-rich red giant stars. However, the graphite identification requires that its structure be well-ordered, which is unlikely in the interstellar radiation environment. Large sheets of polycyclic aromatic hydrocarbons have similar absorption features, but are subject to the same objection. Sakata *et al.*[34] have found a 2,200 Å feature in a hexafluoroisopropanol extract of the Murchison carbonaceous chondrite and attribute its presence to organic molecules with conjugated double bonds of the sort C=C—C=C and C=C—C≡N. But such molecules are abundant in tholins, as in butadiene, heptadiene and butenenitrile. Polysaccharides, on the other hand, exhibit no such feature[35].

The optical region of the interstellar spectrum is noteworthy for the presence of a set of diffuse interstellar lines, the most prominent at 4,428 Å, which have resisted identification for 40 years. The hypothesis that 4,428 Å is the Soret band of porphyrins (tetrapyrroles) is interesting in the light of our identification of pyrrole in spark tholins, but there are serious difficulties with this idea[10]. Douglas[36] has recently argued that the diffuse lines arise from radiationless internal conversion of long-chain C molecules, where the carbon number lies between 5 and 15. Many abundant tholin pyrolysis products satisfy this description. Tholins have a substantial absorption in the blue, responsible for their brown appearance, which is not due to S, and may be caused[2] by conjugated polyenes, H (CH=CH)$_n$ H, where $n \geq 6$. Our pyrolysis fragments[3,4] give good evidence for dienes in both tholins, and longer polyenes seem likely. If tholins are the principal constituents of the interstellar grains, the important conclusion follows that interstellar extinction is not merely due to scattering but must take account of substantial pure absorption as well ($k \sim 10^3$ cm^2 g^{-1} in the violet for UV tholins[2]).

The radii of particles ejected by stellar radiation pressure or stellar winds during the Hayashi or red giant stages of stellar evolution depend on the complex refractive index of the particles, but particles larger than a few μm are unlikely to be ejected. Thus the large tholin particles shown in Fig. 1 would not be ejected, but rather remain in circumstellar space, until degraded by sputtering or other processes into ejection-sized fragments. The smallest particles seen in Fig. 1*d* are, however, <1 μm in diameter and would thus be ejected, but they seem to have the size and at least some of the composition of interstellar grains.

From this general picture, we hypothesise[10] that the organic molecules detected by microwave line spectroscopy are chiefly spallation, spluttering or photodissociation products of tholins in interstellar grains, although UV and cosmic ray-induced positive ion-molecule reactions produce the spheric 1 interstellar organics in dense clouds. Typical lifetimes[40] of icy or dusty grains, unprotected by surrounding interstellar clouds, are 10^8–10^9 years. The interstellar molecules should then resemble the pyrolyzate or MS cracking pattern of the tholins, or the vapourisation products of organics produced by low-temperature UV irradiation of condensates of abundant interstellar gases[11]. In Table 2 this comparison is performed for all identified interstellar molecules composed of H, C, O and N. For simplicity, we have neglected S compounds, but the array of such compounds in appropriate tholins[3] shows that no problems in interpretation are thereby posed. Every identified interstellar molecule either is produced directly in a relevant experiment (75%) or can be produced plausibly from tholin pyrolyzates (25%). Molecules with amino groups are in a special category, since they are degraded to nitriles during pyrolysis; however, the detection of amino acids in our UV tholins, with the very large quantum yield on hydrolysis of $\sim 10^{-4}$, shows[1] that amino groups or their precursors should be present in abundance. There is a tendency (Table 2), perhaps not yet statistically significant, for even C numbers and odd heavy atom numbers to be avoided in high molecular weight interstellar fragments; tholin pyrolyzates do not show such a trend and it will be interesting to see whether microwave lines corresponding to 5, 7, 9 and 11 heavy atoms (such as, methacrylonitrile or toluene) will be found.

The investigation of cometary spectra has now yielded simple nitriles and aldehydes, molecules plausibly derived from cometary tholins formed in the solar nebula[10] and preserved in deep freeze for the intervening 5×10^9 years. The similarities between cometary and interstellar molecules are sufficiently great that searches for a wide range of tholin pyrolyzates and their products seem desirable by remote spectroscopy, but particularly by *in situ* analytic chemistry in a cometary fly-through spacecraft mission. More generally, the tholin fragments identified in the laboratory[3,4] are plausible candidates for future studies of the interstellar medium by UV, visible, IR and microwave spectroscopy. Several molecules (HCN, CH_3CN, HCHO, CH_3CHO, C_2H_5OH) were found[11,37] in such laboratory experiments before their detection in the interstellar medium. The fact that molecules of some considerable complexity are predicted from the tholin argument suggests the need for comprehensive compilations of laboratory spectra of organic molecules, especially in the microwave region. The number of molecules is now so large for a finite microwave spectrum that statistically some molecules identified on the basis of one or two spectral lines may have been identified in error[10].

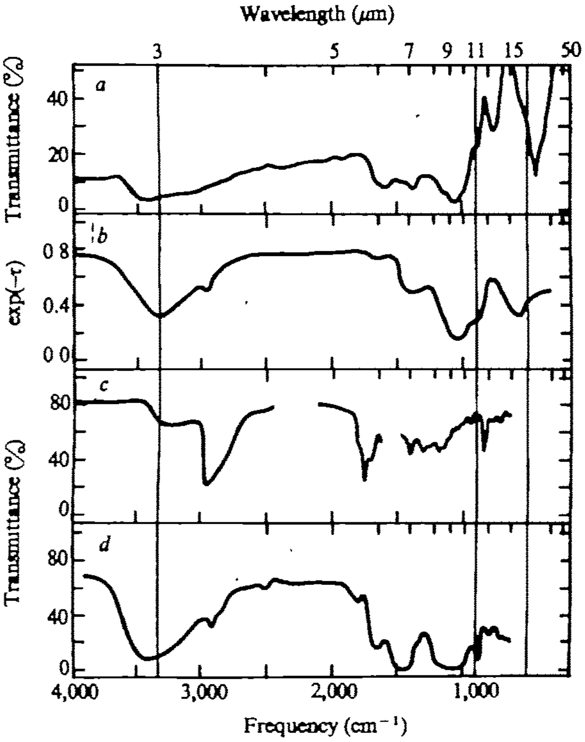

Fig. 4 Comparative IR spectra of *a*, UV tholin mix [Fig. 3 *f*]; *b*, Hoyle's[29] galactic 'best fit', *c*, benzene–methanol extract of the Orgueil carbonaceous chondrite[27,28]; *d*, hot-water soluble, cold-water insoluble extract of the Mokoia carbonaceous chondrite.[27,28]

We believe the connection of this work with the origin and evolution of life on Earth to be indirect, although certainly interesting. The large scale production of complex organics in a preplanetary cloud, and the abundance of simpler organics in so inhospitable an environment as the present interstellar medium, surely imply that prebiological organic chemistry is easy, that much higher quantum yields and synthetic productivities should have applied on the primitive Earth. The presence of cyanoacetylene in the interstellar medium makes its presence, and the copolymerisation of biologically interesting molecules which it induces[34], more likely on the primitive Earth. But the Earth's surface was probably molten in its early history, and organic molecules are unlikely to have survived from the epoch preceding the formation of the Earth, although they should have accumulated thereafter. In any case the synthesis of organics on the primitive Earth seems to have been entirely adequate at least for our present level of understanding of the origin of life[1,10,38]. Surviving exogenous molecules are not needed. Likewise there seems to be no way for indigenous organisms to evolve—the heavy atom collison rate precludes more than about 50 interstellar generations in the age of the Galaxy and the organic reaction rates in cometary nuclei in the Oort cloud should be negligible—much less to arise in the interstellar medium[10] If such organisms did exist[29,39], their pathogenicity on Earth might be an open question, but organisms such as the influenza virus, with a molecular biology entirely typical of indigenous terrestrial organisms, are implausible in the extreme, as extraterrestrial pathogens. There may be a common organic chemistry on countless worlds as in the interstellar medium; but the stochastic nature of the evolutionary process makes it highly improbable that the biochemistry of extraterrestrial organisms will resemble very closely the biochemistry with which we are familiar on Earth.

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Synthesis of rabbit β-globin in cultured monkey kidney cells following infection with a SV40 β-globin recombinant genome

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Rabbit β-globin complementary DNA (cDNA) has been inserted into SV40 DNA in place of the gene coding for the virus' major capsid protein, VP1. The recombinant genome, SVGT5–RaβG, multiplies efficiently in CV1 monkey kidney cell cultures and is transcribed to yield cytoplasmic, polyadenylated hybrid mRNAs containing the β-globin coding sequence. Cells propagating SVGT5–RaβG produce substantial quantities of rabbit β-globin polypeptide.

MAMMALIAN cells infected with SV40 virus carrying cloned eukaryotic genes provide one system in which the expression of eukaryotic genes can be studied[1–3]. SV40 is particularly attractive as a transducing vector for various reasons. The virus genome consists of a single, small, covalently closed circular DNA molecule, whose entire nucleotide sequence has recently been determined[4,5]; the viral DNA is obtainable in large quantities and relatively pure form; the genomic regions responsible for the various viral functions have been accurately located with respect to a detailed physical map of the DNA[4–7]; the viral genome can multiply vegetatively or as an integral part of cellular chromosomes; and a wealth of information exists on the replication and expression of the viral genome and their regulation in different host cells.

To date a number of genes have been recombined *in vitro* with SV40 vectors and propagated in cultured monkey kidney cells; these include segments of λ phage DNA[1,2], *Escherichia coli* DNA coding for tRNA^{Tyr} (ref. 3), thymidine kinase (S. P. Goff and P. Berg, in preparation), guanine phosphoribosyl transferase (R. C. Mulligan and P. Berg, unpublished) and *Drosophila melanogaster* DNA containing the genes for H2A and H4 histones (H. Hofstetter and P. Berg, unpublished). Although the exogeneous DNA segments are transcribed during infections with each of these recombinant genomes, discrete, mature functional mRNAs have not been detected.

We report here the construction and propagation of a recombinant genome in which virtually all of the coding sequence for the major capsid protein, VP1, located between map coordinates 0.945 and 0.145 (see Fig. 1*a*) has been replaced by a cDNA that specifies the entire amino acid sequence of rabbit β-globin[8,9]. Cells infected with the recombinant genome

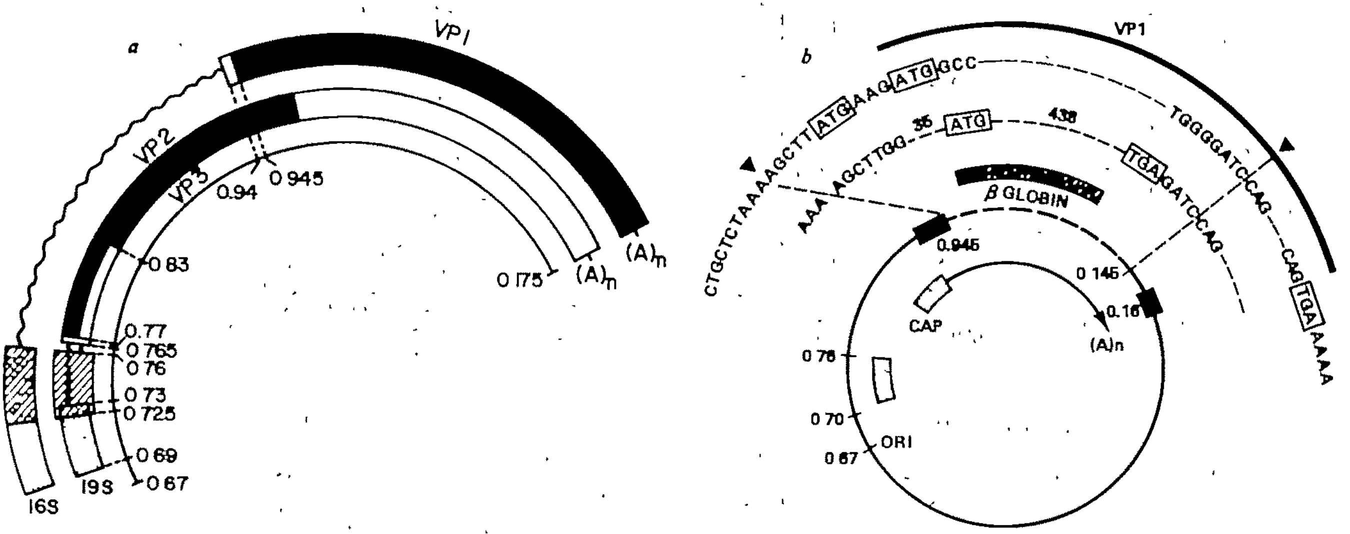

Fig. 1 *a*, The composite structure of SV40 late mRNAs. The map of SV40 DNA from the origin of DNA replication (0.67) to the end of the late region (0 175) is shown on the inner circle The regions coding for the structure of VP1, VP2 and VP3 appear as shaded regions within bars that define the 'bodies' of the 19S and 16S mRNAs. The 'leader' segments are shown as bars spanning map coordinates 0.69–0.76. The cross-hatched and stippled regions of the 16S mRNA leader, for example, are intended as symbolic, rather than literal representations of more than one type of leader segment; one leader segment spans the region from 0.725 to 0.76 ▩ and another the region from 0 69 to 0 76 ☐. For the 19S mRNA species, one class of leaders spans map coordinates 0 69 to 0 73 ☐ and is joined to the body at 0 76; another class of leaders extends from 0 725 to 0 76 ▩ *b*, SV40 and rabbit β-globin cDNA nucleotide sequences relevant to the construction of SVGT5–RaβG The origin of SV40 DNA replication at map position 0.67 is denoted as ori. SV40 16S late mRNA, with its 'capped' 5′ leader sequence derived from 0.72 to 0.76 map units (stippled box), and 3′ poly(A), is represented inside the circle. The region of the map coding for VP1 is represented by the heavy black line labelled VP1. The regions of SV40 DNA implicated in splicing and polyadenylation of the 16S mRNA are shown as black rectangles on the circular map. The dashed line extending leftward from map position 0 945 indicates the position of a *Hin*dIII endonuclease cleavage site in SV40 DNA (the outer sequence) and in pBR322–βG2 DNA (the inner sequence) The dashed line extending rightward from map position 0.145 locates the unique *Bam*HI restriction site of SV40 DNA (outer sequence) and the *Bgl*II endonuclease cleavage site of pBR322–βG2 (inner sequence). The nucleotide sequences between these dashed lines show the β-globin cDNA sequences inserted into SVGT5 and the SV40 sequences excised to construct SVGT5 The triplets enclosed in boxes show the initiation and termination signals for translation of VP1 and β-globin. The β-globin cDNA sequence in pBR322–βG2 contains 35 nucleotides proximal to the initiator AUG in β-globin mRNA The small hatched bar spans the region containing the initiation β-globin codon, the 438 nucleotides specifying the entire β-globin protein amino acid sequence, and the translation terminator codon

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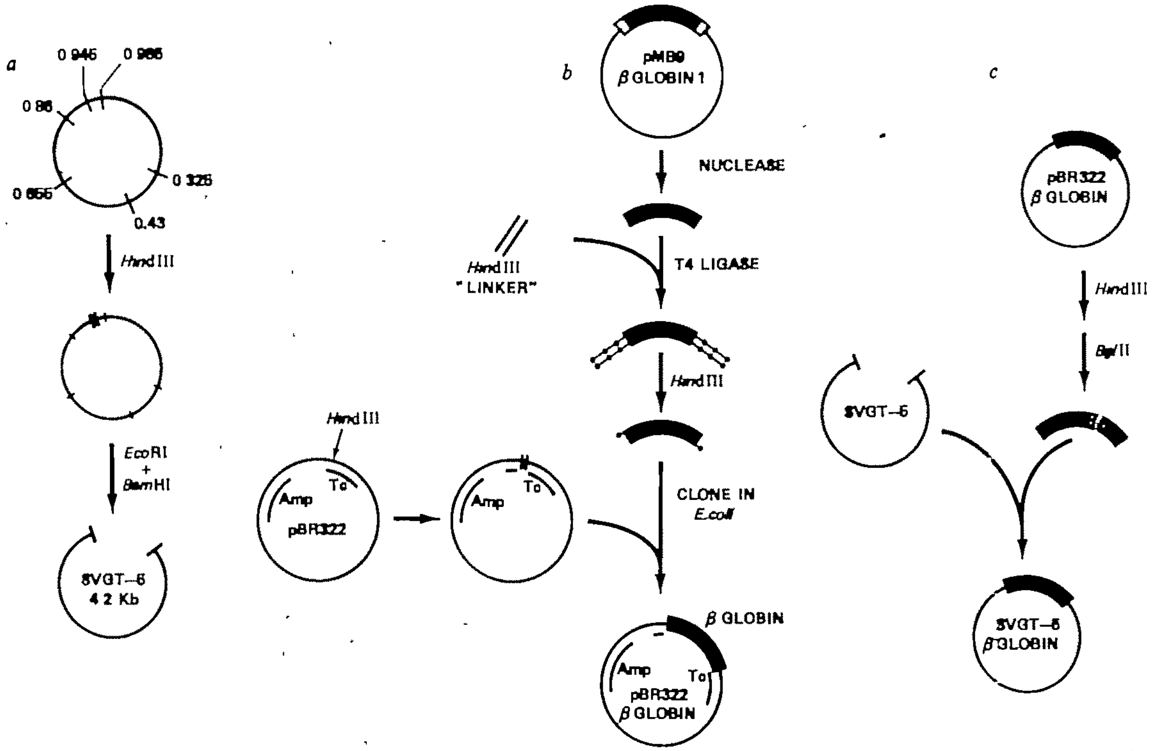

Fig. 2 Construction of SV40-Raβ G recombinant genome *a*, Construction of SVGT5 vector. Approximately 100 μg of SV40 DNA were incubated with 100 U of *Hin*dIII endonuclease (Boehringer) for 50 min at 30 °C in 10 mM Tris, *p*H 7.5, 5 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT The partial digest was electrophoresed on a 0.8% agarose gel in Tris–borate buffer [6] to obtain full-length linear DNA molecules The full-length linear DNA band was eluted electrophoretically from an excised gel slice, digested with an excess of *Bam*HI endonuclease (N E. Biolabs) in 6 mM Tris, *p*H 7.5 containing 6 mM β-mercaptoethanol and 6 mM $MgCl_2$, then, after adjusting the Tris to 100 mM, the mixture was digested with an excess of *Eco*RI endonuclease (N E Biolabs). The digest was electrophoresed on a 0.7% agarose gel and the largest band (4.2-kilobase) was excised and electroeluted To remove residual agarose, the DNA was absorbed to a 0.5 ml DE-52 column equilibrated with TE (10 mM Tris, *p*H 7.5, 1 mM EDTA), after washing the column with TE the DNA was eluted with TE containing 1 M NaCl. *b*, Subcloning of β-globin cDNA PβG1 DNA was incubated with 1,200 U of S_1 nuclease (Miles) for 30 min at 50 °C in buffer containing 30 mM sodium acetate (*p*H 4.5), 1 mM $ZnSO_4$, 0.2 M NaCl and 45% (w/v) formamide[17]. The digestion was terminated by the addition of 10 mM EDTA, the DNA was precipitated with ethanol and electrophoresed in a 5% acrylamide gel in Tris–borate buffer for 3 h at 125 V. The ethidium bromide stained band containing the β-globin cDNA (0.5–0.58 kilobases) was excised and electroeluted One μg of the β-globin cDNA was incubated with 3 μg *E.coli* polymerase I (provided by A. Kornberg) in 25 μl containing 60 mM Tris, *p*H 7.5, 8 mM $MgCl_2$, 10 mM β-mercaptoethanol, 1 mM ATP, and 0.2 mM of each of the deoxynucleoside triphosphates for 10 min at 12 °C to convert 'ragged' into 'blunt' ends[16] ^{32}P-labelled CCAAGCTTGG oligonucleotide (obtained from Collaborative Research) was added in a 50-fold molar excess relative to the cDNA termini and the reaction adjusted to contain 1 mM ATP. T4 DNA ligase (Bethesda Research Laboratory) (50 U ml^{-1}) was added, the mixture (50 μl) incubated for 1 h at 20 °C and then heated at 80 °C for 5 min After adjusting the salt concentration to 50 mM, *Hin*dIII endonuclease (15 units) was added and after 4 h at 37 °C, the digest was electrophoresed on a 5% acrylamide gel The β-globin cDNA band was located by autoradiography, excised, and electroeluted Approximately 0.7 μg of the recovered DNA was mixed with 1.2 μg of *Hin*dIII endonuclease-cleaved pBR322 DNA in 50 mM Tris buffer, *p*H 7.8, containing 10 mM $MgCl_2$, 20 mM DTT, and 1 mM ATP (ligase buffer), 5 units of T4 DNA ligase were added and after an overnight incubation at 12 °C, suitable dilutions of the ligated mixture were introduced into HB101 (ref. 20). Transformants were selected on agar medium containing 20 μg ml^{-1} ampicillin. Approximately 10^4 Amp^r transformants were obtained per μg of ligated DNA. Amp^r colonies were picked onto agar medium containing 50 μg ml^{-1} tetracycline to identify recombinant plasmids with insertions at the *Hin*dIII restriction site[21]. Ten of 22 Tet^s clones carried plasmids containing the β-globin insert One isolate pBR322–βG2, served as the source of β-globin cDNA for insertion into SV40 DNA *c*, Construction of SVGT5–RaβG. pBR322–βG2 DNA was cleaved sequentially with an excess of *Bgl*II and *Hin*dIII endonucleases in the buffers and conditions described above. The products were electrophoresed on a 4% acrylamide gel and the 485-base pair β-globin cDNA band was excised and electroeluted. Approximately 12 ng of the cDNA were incubated with 100 ng of SVGT5 DNA and T4 DNA ligase (25 U ml^{-1}) in ligase buffer overnight at 12 °C. Dilutions of the ligated DNA and *ts*A58 DNA were used to transfect CV-1P cells as already described[22] Fifty plaques were picked and (their viral DNAs) screened for the presence of β-globin cDNA sequences by a modification of the plaque hybridisation method of Villarreal and Berg[23]. A plate of confluent CV-1P cells (100 cm) was overlaid with 40 ml of agar medium and wells, 0.5 cm in diameter, were punched to expose small areas of the cell monolayer. An aliquot (10 μl) of each plaque suspension was added to a well, and after a 2 h incubation at 37 °C, the wells were filled with agar medium After 3 d at 41 °C, the plate was overlaid with agar medium containing 0.01% Neutral Red and incubated overnight at 39°C The agar layer was removed and the cell monolayer transferred to a nitrocellulose disk[23]; the disk was annealed with [^{32}P]-pBR322–βG2 DNA, washed and autoradiographed Forty-six of the 50 plaques picked showed unmistakable evidence of hybridisation, thereby indicating the presence of β-globin cDNA sequences

(SVGT5–RaβG) produce substantial quantities of discrete hybrid mRNAs that contain the β-globin coding sequence flanked by 5′–'leader' and 3′–terminal sequences characteristic of late SV40 mRNAs. The infected cells also synthesise rabbit β-globin polypeptide in quantities nearly equivalent to the amount of VP1 produced by the co-infecting helper virus.

Construction of SVGT5–RaβG

The mRNAs which code for SV40's three capsid proteins, VP1, VP2 and VP3 are composite structures, that is, the mRNAs contain nucleotide sequences transcribed from non-adjacent DNA segments[10,11]. Each mRNA contains a 5′–terminal segment (the leader) transcribed from the l-strand between map coordinates 0.69 and 0.76 (see Fig. 1*a*). Joined to the leader sequence, the VP2 and VP3 mRNAs (the 19S class) have a segment of RNA homologous to the l-strand between map coordinates 0.77 and 0.175 (the 19S body); the body of the VP1 mRNA (16S class) is transcribed from the l-strand of the DNA between map coordinates 0.94 and 0.175.

Deletion mutants have been obtained which lack the 3′ boundary of the late region leader segments[12]. RNA transcribed from these mutants is not processed into mature 16 or 19S mRNA, presumably, because splicing cannot occur. Previously constructed SV40 vectors resemble these deletion mutants in that they have lost most or all sequences required for splicing. Very possibly the failure to obtain discrete mRNAs with the earlier recombinants stems, at least in part, from the consequent disruption of post-transcriptional splicing reactions. We have therefore sought to construct a SV40 vector which would retain all the regions implicated in transcriptional initiation and termination, splicing and polyadenylation of 16 and 19S mRNAs (see Fig. 1*a*).

Inspection of the SV40 restriction map suggested two restriction endonuclease-cleavage sites that could be used to generate such a vector. The first, a *Hin*dIII restriction site at map position 0.945, is six nucleotides proximal to the initiation codon for VP1 (refs 4,5) and about 50 nucleotides distal to the site at which the leader sequence is joined to the body of the VP1 mRNA[13,14] (see

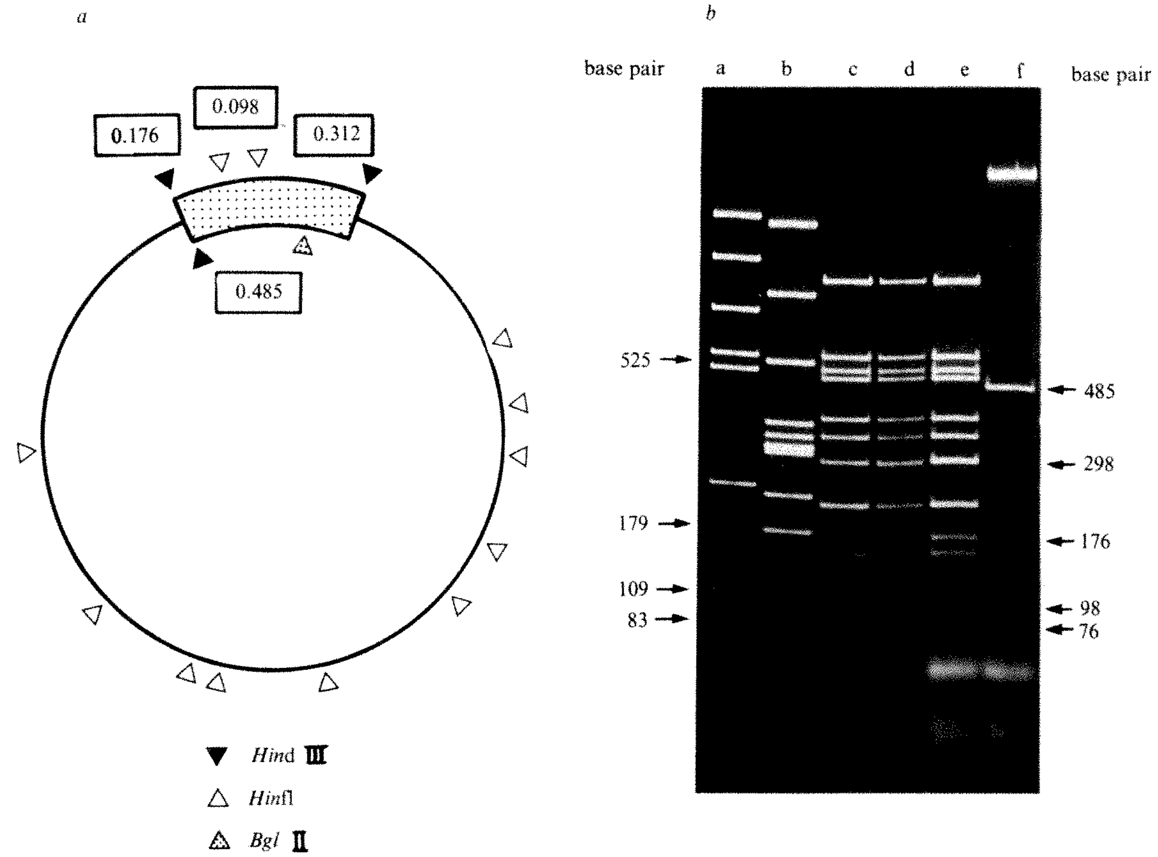

Fig. 3 Restriction analysis of pBR322–βG recombinants. *a*, The numbers in boxes are the expected sizes of restriction fragments, assuming the recovery of the complete cDNA sequence present in PβG1. The stippled region denotes the position of the β-globin cDNA insert and the various symbols indicate the particular restriction endonuclease cleavage sites. *b*, pBR322–βG DNAs were isolated as described by Katz *et al.*[40], digested with an excess of the indicated enzymes and electrophoresed on a 3–7% gradient acrylamide gel[6]. The gel was stained with ethidium bromide (1 μg ml^{-1}) and photographed under short wave ultraviolet light, using Polaroid 665 film and an orange filter. a, *Hin*fI endonuclease digested SV40 DNA. b, *Hae*III endonuclease digested SV40 DNA. c–e, *Hin*dIII plus *Hin*fI endonuclease digested pBR322, pBR322–βG2, pBR322–βG3 DNAs, respectively. f, *Hin*dIII plus *Bgl*II digested pBR322–βG2 DNA.

Fig. 1*b*). The second, a *Bam*HI restriction site at map coordinate 0.145, is 50 nucleotides proximal to the termination codon for VP1 translation and 150 nucleotides before the poly(A) sequence at the 3′-end of VP1 mRNA[4]. The 4.18 kilobase DNA segment bounded by these two restriction sites (SVGT5) contains, in addition to the regions at which splicing and polyadenylation of late mRNAs occur, the origin of DNA replication and the entire early region; hence, it can be propagated by complementation with an appropriate *ts*A mutant[15].

The rabbit β-globin cDNA sequence was selected as the transducible gene because it is small enough to be accomodated in SVGT5 and because the protein can be isolated and characterised readily. Furthermore, the cDNA had already been cloned in *E. coli* and shown to contain the complete coding sequence for β-globin, as well as additional 5′ and 3′-terminal nucleotide sequences present in β-globin mRNA[9]. Our aim was to excise the β-globin cDNA segment from pMB9, modify and reclone it in another *E. coli* plasmid, pBR322 (ref. 16); in doing this we could obtain the β-globin cDNA segment with a *Hin*dIII cohesive end just proximal to β-globin's initiator codon (see Fig. 1*b*). Consequently, SVGT5 and the modified β-globin cDNA segment could be joined readily to form the desired recombinant genome.

Construction, propagation and characterisation of SVGT5–RaβG genomes

To obtain SGVT5, wild-type SV40 DNA was partially digested with *Hin*dIII endonuclease to produce full-length linear molecules (Fig. 2*a*). Full-length linear DNAs were isolated by electrophoresis in agarose gel and digested with *Bam*HI and *Eco*RI endonucleases. These cleavages, and the subsequent electrophoretic separation, yielded a SV40 DNA fragment of 4.18 kilobases which contained the nucleotide sequences between map coordinate 0.145, clockwise to the *Hin*dIII restriction site at map coordinate 0.945. The *Eco*RI endonuclease digestion was introduced to eliminate a potentially contaminating fragment of 4.28 kilobases formed by cleavages at map position 0.325 (a *Hin*dIII restriction site) and the *Bam*HI restriction site at 0.145.

The rabbit β-globin coding sequence was isolated from the PβG1 plasmid recombinant described by Maniatis *et al.*[8].PβG1 was obtained by inserting a cDNA copy of purified rabbit β-globin mRNA at the *Eco*RI restriction site of the plasmid pMB9 using the poly(dA):(dT) joining method, and propagating the recombinant plasmid in *E. coli* (strain HB101)[8]. Nucleotide sequence analysis confirmed that PβG1 contained the entire β-globin coding sequence, all of the 3′ and most (43 of 56 bases) of the 5′-non-coding sequences in β-globin mRNA[9].

PβG1 DNA was digested with S_1 nuclease as described by Hofstetter *et al.*[17] to excise the β-globin cDNA segment from PβG1 (Fig. 2*b*). β-Globin cDNA fragments varying in size between 500–585 base pairs were recovered by electrophoresis in acrylamide gels and incubated with *E. coli* DNA polymerase I and the four deoxynucleoside triphosphates to convert 'ragged' into 'blunt' ends[16]. A decanucleotide 'linker', CCAAGCTTGG, containing the *Hin*dIII endonuclease recognition sequence, was phosphorylated at its 5-end with polynucleotide kinase and $[\gamma-{}^{32}P]ATP$[18] and then joined to the ends of the β-globin cDNA with T4 ligase[19]; subsequent digestion with *Hin*dIII endonuclease generated *Hin*dIII cohesive ends.

The β-globin cDNA with its *Hin*dIII cohesive ends was ligated to *Hin*dIII endonuclease-cleaved pBR322 with T4 DNA ligase, and introduced into *E. coli* K12 (strain HB101)[20]. Transformants were selected for ampicillin resistance and pBR322–βG recombinants were screened for their sensitivity to high levels of tetracycline. (Cells containing pBR322 are resistant to ampicillin and tetracycline, but insertions at the *Hin*dIII restriction site eliminate resistance to high levels of tetracycline[21].) Small cultures of each putative pBR322-βG recombinant were grown, the plasmid DNA was isolated, cleaved with *Hin*dIII endonuclease and the products electrophoresed in agarose; 10 of 22 isolated plasmid DNAs yielded fragments 0.50 to 0.58 kilobases in addition to the linear pBR322 DNA (data not shown).

One of these, pBR322-βG2, produced an insert fragment of about 570 base pairs following *Hin*dIII endonuclease digestion. Two fragments, 485 and 76 base pairs long were detected by digesting this plasmid with *Hin*dIII and *Bgl*II endonucleases (Fig. 3 track f). These findings show that the expected *Hin*dIII joins are intact, that they are separated from each other by nearly the expected length of the β-globin cDNA segment and that the single internal *Bgl*II restriction site in the β-globin cDNA is present. These conclusions are reinforced by the double digestions of pBR322-βG2 and pBR322-βG3 with *Hin*dIII and *Hin*fI endonucleases (Fig. 3 tracks d and e). Fragments corresponding to the expected 176 base pair fragment (derived from the 5′-end of the β-globin coding sequence) and 98 base pair fragment (produced from an internal sequence in the cDNA) were readily apparent in the electropherogram. The predicted 312-base pair fragment, generated from the 3′-end of the globin coding sequence, is actually 298 base pairs and is obscured by a fragment of the same size derived from the vector DNA. This was deduced by inspection of the original film in which the staining of the 298-base pair band was more intense than the band migrating behind it. Determination of the nucleotide sequences[18] at the two *Hin*dIII join sites in pBR322-βG2 further supports the restriction enzyme analysis and establishes that 5 nucleotides at the 5′-end, 15 nucleotides at the 3′-end and all of the dA:dT sequences of the originally cloned β-globin cDNA are absent in the β-globin cDNA segment recloned in pBR322 (data not shown).

The β-globin cDNA segment of pBR322-βG2 was excised by sequential digestion with *Hin*dIII and *Bgl*II endonucleases (Fig. 2*c*). This yielded a fragment having the codon for initiating translation of β-globin 37 nucleotides beyond the *Hin*dIII endonuclease-generated cohesive end and the translation termination codon just proximal to the *Bgl*II endonuclease-generated cohesive end. Even though the *Bam*HI (GGATCC) and *Bgl*II (AGATCT) endonuclease recognition sites differ, the cohesive ends generated by the two endonucleases are identical (GATC). Accordingly, the 0.485-kilobase β-globin cDNA fragment could be ligated to SVGT5 with T4 ligase[24] (Fig. 2*c*). To propagate the SVGT5-βG2 genome, CV1-P cells were transfected with a mixture of the ligated DNA and *ts*A58 DNA[22]. *ts*A58 is a thermosensitive mutant of the SV40 early function and can not multiply at 41 °C (ref. 15). About 2.5×10^4 plaques were obtained per microgram of ligated DNA.

These plaques were screened for virus containing the β-globin cDNA using a modification of the *in situ* plaque hybridisation technique[23](see legend to Fig. 2*c*). Forty-six of fifty plaques examined contained DNA that hybridised specifically to the β-globin cDNA. Recombinant virus from independent clones were purified by plaque isolation and high-titre virus stocks and purified viral DNA were prepared in CV1 cells. Judging from the relative yields of the two DNAs from such mixed infections, we estimate that the recombinant and helper genomes multiply equally well.

As expected from its somewhat smaller size (4.7 compared with 5.2 kilobases), SVGT5-βG DNA is separable from the helper *ts*A58 DNA by electrophoresis in agarose (Fig. 4, tracks a–c). Restriction endonuclease digestions and electrophoresis of the products helped to confirm the structure of the hybrid DNA. The locations of the *Hin*dIII and *Mbo*I restriction sites in SVGT5 and the β-globin cDNA sequence are indicated in Fig. 4*a*. Correct joining of the β-globin cDNA to SVGT5 regenerates the *Hin*dIII restriction site at map position 0.945. Therefore, digestion of SVGT5-βG DNA with *Hin*dIII endonuclease should generate a fragment of 1.43 kilobases from cleavages at the *Hin*dIII join sequence and the *Hin*dIII restriction site at map position 0.325 in the vector; the 1.43-kilobase fragment is produced in the *Hin*dIII endonuclease digests of two independent recombinants, SVGT5-βG 10A and SVGT5-βG 12A (Fig. 4, tracks e and f), but is absent from a comparable digest of SV40 DNA (track d). After transfer of the DNA in the gel to a nitrocellulose sheet[25] and hybridisation with [^{32}P] PβG1

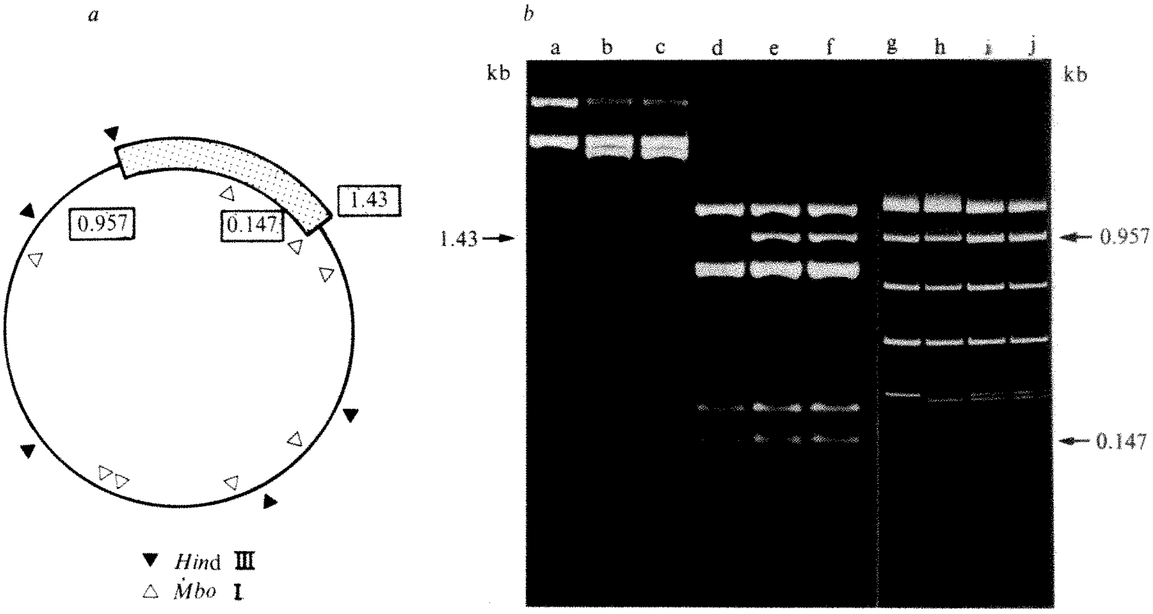

Fig. 4 Restriction analysis of SVGT-RaβG. *a*, The stippled region denotes the β-globin cDNA sequence and the numbers enclosed in boxes are the expected fragment sizes based on the sequence of the β-globin cDNA in pBR322–βG2 and the known locations of the *Hin*dIII and *Mbo*I restriction sites. *b*, Viral DNA was extracted by Hirt's method[27] from CVl cells that had been infected with plaque-purified SVGT5–βG and *ts*A58 virus (M.O.I. 0.005 PFU per cell). The DNA was centrifuged to equilibrium in ethidium bromide–caesium chloride gradients[28]; aliquots were then digested with the appropriate enzymes and electrophoresed on either 0.8% agarose gels (tracks a–f) or on 3–7% gradient acrylamide gels (tracks g–j). Tracks a–c: undigested SV40, SVGT5–βG10A and SVGT5–βG12A DNAs. Tracks d–f: the same three DNAs digested with *Hin*dIII. Tracks g–h: *Mbo*I endonuclease digests of SV40, *ts*A58, SVGT5–βG10A and SVGT5–βG12A DNAs.

DNA, only the 1.43-kilobase fragment was labelled (data not shown); hence, all of the β-globin cDNA sequence is contained within that fragment.

Ligation of *Bam*HI and *Bgl*II endonuclease-created cohesive ends should generate a sequence which is resistant to cleavage by these two enzymes but sensitive to *Mbo*I endonuclease (recognition sequence NGATCN). Indeed, *Mbo*I endonuclease digestion of SVGT5-βG 10A and SVGT5-βG 12A DNAs produced the expected array of fragments (Fig. 4*b*). The 0.957 fragment, which spans the *Hin*dIII join site, comigrates with one of the vector DNA fragments (compare tracks i and j with g and h); the 0.147-kilobase fragment, formed from cleavages at a *Mbo*I restriction site within the β-globin cDNA and the *Mbo*I site formed by the join of the *Bgl*II and *Bam*HI cohesive ends is produced from both recombinants (tracks i and j) but is absent from SV40 DNAs (tracks g and h). Note that the 0.240 fragment produced from the SV40 DNA used to make the vector (track g) is slightly larger than the corresponding fragment cleaved from the *ts*A58 helper DNA (track h). The mixture of recombinant and helper DNA yielded both fragments in about equal amounts indicating that here too the recombinant and helper genomes were equally represented in the infection from which these DNAs were isolated.

Although not shown, 8 of 14 other independent clones of SVGT5-βG gave restriction digests comparable to the two documented here. These data indicate that the β-globin cDNA cloned in pBR322 has been introduced into SVGT5 in the expected way.

Formation of SV40-Ra β-globin hybrid mRNAs

Considering the structure of SVGT5-βG and present ideas about SV40 late mRNA formation, two cytoplasmic SV40–β-globin hybrid mRNAs should be formed following infection with SVGT5-βG. These should be about 0.5 kilobases smaller than the normal SV40 19S and 16S late mRNAs. To test these expectations, CV1 cells were infected with *ts*A58 and each of two SVGT5-βG recombinant viruses (MOI 10–50 PFU per cell) at 37 °C and, after 48 h, cytoplasmic, poly(A)containing RNA (freed from DNA) was isolated (see legend to Fig. 5); similar RNA preparations were isolated from SV40 and mock-infected cultures. Each RNA preparation was denatured with glyoxal[26], electrophoresed on 1.5% agarose gel and transferred to diazotised benzyloxymethyl paper[12,30]. The imprints were annealed with ^{32}P-DNA to reveal the positions of the separated RNAs (Fig. 5).

With [^{32}P] PβG1 DNA as hybridisation probe, two discrete β-globin mRNA species are detectable in the RNA from cells infected with either recombinant (Fig. 5, tracks c and d). These RNAs are missing in the RNA from mock or SV40-infected cells (tracks a and b). With DNA fragments of known size as length standards (tracks e and f) we estimate the sizes of these mRNAs as 1.8 and 1 kilobases. When duplicate tracks were hybridised with [^{32}P]-SV40 DNA containing the late mRNA leader sequences, two labelled bands are visible in the SV40-infected cell RNA (track i), but not in the RNA from mock-infected cells (track j). The faster migrating, more heavily labelled band is the 16S mRNA (1.5 kilobase) and the slower, less heavily labelled band the 19S mRNA (2.3 kilobase). There are four labelled bands when RNA from cells infected with the two SVGT5-βG recombinants is hybridised with the labelled SV40 leader probe (tracks g and h). Two (the 1.5 and 2.3-kilobase species) are the 16S and 19S mRNAs produced by the *ts*A58 helper genome. Another has the mobility of the 1-kilobase RNA species that hybridised to the globin cDNA probe. The fourth, which is only barely discernable in this exposure, has a mobility very close to that of the 1.8-kilobase RNA detected with the β-globin cDNA probe. Thus the 1 and 1.8-kilobase polyadenylated RNAs contain the β-globin cDNA sequence and the leader segment characteristic of SV40 late mRNAs. Comparing the labelling intensities of the 1-kilobase β-globin mRNA and the 1.5-kilobase SV40 mRNA (in Figs, tracks g and h) and taking account of the ratio of recombinant to helper DNA in these infected cultures (0.3 recombinant), we surmise that the steady state levels of the two mRNAs are within a factor of two of each other.

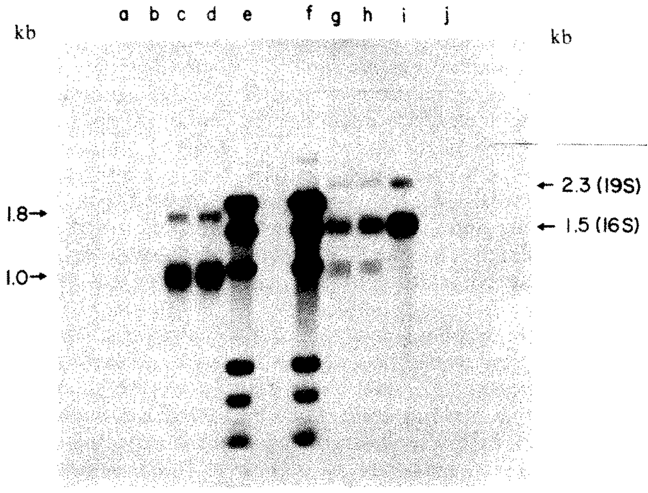

Fig. 5 Analysis of mRNAs made in cells infected with SVGT5-βG. Cytoplasmic poly(A)-containing RNA was isolated from CV1 cells 48 h after infection with a mixture of SVGT5-βG and *ts*A58 virus (>10 PFU per cell) as follows. Cells were washed twice with ice-cold Tris-buffered saline and once with a cold solution containing 10 mM Tris, *p*H 7.5, 5 mM $MgCl_2$, 10 mM NaCl. The cell monolayer, in the latter buffer containing 0.1% NP40 and 0.05% deoxycholate (1 ml per plate), was scraped off the plate with a rubber policeman and centrifuged for 2 min at 1,500 r.p.m. to pellet the nuclei. The supernatant was mixed with an equal volume of solution containing 0.3 M NaCl, 0.1 M Tris, *p*H 8.0, 10 mM EDTA, and 0.15% SDS. Cell aggregates were dispersed by pipetting and the suspension was extracted two times with an equal volume of water-saturated phenol-chloroform (1:1). After ethanol precipitation, the nucleic acid was digested with iodoacetate-treated DNase[29] (Worthington) (20 μg per mg nucleic acid), at room temperature for 45 min in a small volume of 20 mM Tris, *p*H 7.5, 10 mM NaCl, and 10 mM $MgCl_2$. The mixture was extracted twice with phenol-chloroform and passed over poly(U)-Sepharose[41] to isolate poly(A)-containing RNA. Aliquots of the poly(A)-containing RNA were denatured with glyoxal[26] and electrophoresed in a 1.5% agarose gel in 10 mM sodium phosphate *p*H 7.0. The fractionated RNA was then transferred to diazotised benzyloxymethyl paper[30] as described by Villarreal, White and Berg[12]. The paper was annealed with about 4×10^6 c.p.m. of the indicated ^{32}P-nick-translated DNA probe for 24 h, then washed[12] and autoradiographed for 2 d. ^{32}P-labelled pBR322-βG2 DNA fragment (from map position 0.67–0.76, produced by cleavages with *Bgl*I and *Hpa*I endonucleases) was the hybrisation probe for tracks g–j. Tracks e and f contained a *Hin*dII digest of [^{32}P] SV40 DNA as size standards. The lengths of the fragments (kilobases) in decreasing order are: 1.96, 1.54, 1.07, 0.369, 0.240. Tracks a, j; RNA from non-infected cells; b, i SV40 RNA; c, h SVGT5-βG10A RNA; d, g SVGT5-βG12A RNA.

Formation of β-globin

One goal of our experiment was to determine whether replacing the VP1 coding sequence with the β-globin sequence would permit the formation of a discrete, cytoplasmic SV40–β-globin hybrid mRNA. An additional goal was to determine whether such SV40-β-globin hybrid mRNAs could be translated *in vivo* to produce β-globin. CV1 cells were infected with *ts*A58 and either of the two SVGT5-βG recombinants (MOI 10-50 PFU per cell) and, after 48 h (37 °C), the cultures were labelled with ^{3}H-leucine and ^{3}H-valine for 30 min; SV40 and mock-infected cultures were labelled in a comparable way. An extract of each culture was prepared by sonication and treatment with NP40-deoxycholate; aliquots were electrophoresed on a SDS 15–20% gradient acrylamide gel (see legend to Fig. 6). The autoradiograms of each electrophoresed extract had a complex array of labelled protein bands (Fig. 6 tracks a–d). But the patterns from the SVGT-βG infected cell extracts (tracks c and d) contain a protein band migrating at the position of added rabbit β-globin; this protein band is not discernable in either the SV40 or mock-infected cell extracts (tracks a and b). The formation of a β-globin-like protein in cells infected with SVGT5-βG is supported by the fact that a protein with the same mobility as authentic rabbit β-globin can be recovered from complexes formed by incubating the cell extracts with purified goat anti-rabbit β-globin immunoglobulin (kindly provided by S. Boyer, Johns Hopkins) and heat killed, formalin-fixed *Staphylococcus aureus* cells[33]; the electrophoretic pattern of the labelled proteins eluted with SDS and urea from such complexes are shown in tracks e–h. β-globin bands are seen in the extracts from cells infected with the recombinant (tracks g and h), but not in extracts from uninfected and SV40-infected cells (tracks e and f). If the extracts from SVGT5-βG infected cells are mixed with non-immune goat serum (tracks i and j) or anti-SV40 VP1 serum (track k), before adding the *S. aureus* cells, no β-globin is detectable in the electrophoresed samples.

In a preliminary experiment (carried out in collaboration with T. Hunter at the Salk Institute) the putative β-globin protein obtained from cells infected with SVGT5-βG was immunoprecipitated with the immune serum and *S. aureus* cells mentioned above, eluted with SDS, digested with trypsin and analysed by the two-dimensional electrophoretic-chromatographic procedure[36]. The tryptic peptide maps of the putative β-globin and labelled rabbit β-globin synthesised *in vitro*[36] were strikingly similar; at least 8 of 10 peptide spots in the two maps matched (data not shown). Further experiments to characterise the β-globin, particularly the amino and carboxy terminal amino acid sequences, are in progress.

In experiments to be published later we have compared the amounts of β-globin and VP1 synthesised in cells infected with SVGT5-βG and the helper virus *ts*A58. ^{35}S-methionine labelled infected cell extracts were mixed with either anti-β-globin or anti-SV40 VP1 serum, both immune complexes were adsorbed to *S. aureus* cells, washed, eluted with SDS, electrophoresed in SDS–polyacrylamide gel and autoradiographed. The amount of ^{35}S-label in β-globin and VP1 was quantitated by densitometry of their respective bands in the autoradiograms. Taking into account the difference in the number of methionine residues per mole of β-globin and VP1 (1 : 9) and the ratio of SVGT5-βG to *ts*A58 genomes during the period of protein labelling (1 : 3), we estimate that β-globin and VP1 are synthesised at nearly equal rates.

Discussion

These experiments establish that an exogenous gene, in this case the coding segment for rabbit β-globin, can be recombined with SV40 DNA *in vitro* to yield a hybrid genome that expresses the incorporated gene. The principal conceptual innovation is the decision to leave intact the regions in the vector implicated in SV40 late mRNA processing: specifically, the late mRNA leader sequence, the sequence to which the leader is spliced during maturation of late mRNAs, and the region in which late transcripts terminate and are polyadenylated (see Fig. 1*a* and *b*). In the recombinant genome, virtually all of the coding sequence of SV40's major capsid protein VP1 has been replaced by a β-globin cDNA. β-globin's initiator codon, and presumably its ribosome-binding sequence, replace the corresponding initiation sequence of VP1; furthermore, β-globin's translation terminator codon is proximal to the VP1 termination codon remaining in the vector (Fig. 1*b*). This strategy was adopted so that the β-globin coding sequence would be incorporated into mature SV40-β-globin hybrid mRNAs and be translated.

The success of the experiment clearly demonstrates that processing of both the 16 and 19S late mRNAs does not require nucleotide sequences within the VP1 coding region: analogous (smaller) mRNAs with poly(A) and leader sequences are formed efficiently when most of the VP1 coding sequence is replaced by the β-globin cDNA. Detailed studies are needed to compare the nucleotide sequences of wild-type 16 and 19S mRNA leaders with SVGT5-βG's corresponding β-globin mRNA leader sequences; it may also be interesting to compare the relative rates of the various splicing reactions of normal and recombinant RNAs. Another intriguing question is whether the monkey cells can excise the two intervening sequences present in the rabbit β-globin genomic DNA segment (Maniatis, T. *et al.*, in preparation), a question which can be examined with recombinants in which the rabbit β-globin genomic DNA sequence has been inserted into analogous SVGT vectors.

VP1 is believed to be translated exclusively from the 16S mRNA although the protein's coding sequence is also contained within the 19S mRNA[37]. Therefore, β-globin is probably translated from the 1-kilobase SV40-β-globin hybrid mRNA (the 16S mRNA analogue) and not the 1.8-kilobase hybrid mRNA (the 19S mRNA analogue). Conceivably, however, β-globin may be translated from the 19S mRNA analogue as well, either because the altered nucleotide sequence of the large hybrid mRNA significantly affects its secondary structure or because the ribosome binding site for β-globin is functionally independent of its position within a message. Experiments are in progress to insert the β-globin coding sequence at several different locations in the late and early regions of SV40 DNA, making it possible to assess the effect of varying the distance of the β-globin coding sequence from the 5'-end of the mRNA, the role of neighbouring sequences and any regulatory influence on the efficiency of expression—transcription, processing and translation—of the β-globin 'gene'.

Of particular interest is whether other genes can be expressed if they are inserted into appropriate SV40 DNA vectors in a similar way. Hofstetter and Berg (unpublished results) have

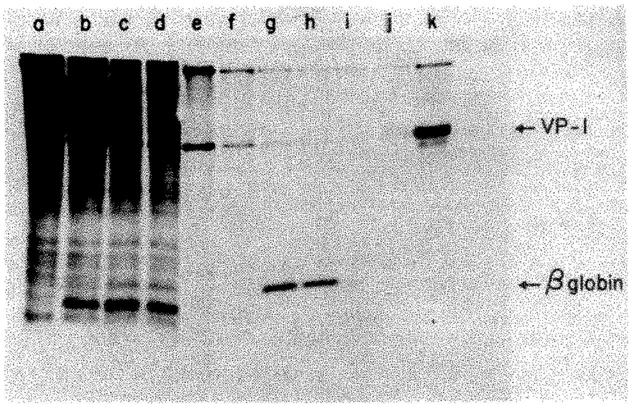

Fig. 6 Production of rabbit β-globin in cells infected with SVGT5-RaβG. Subconfluent CV1 cells were infected with a mixture of SVT5-βG and *ts*A58 (>10 PFU per cell) for 48 h at 37 °C in a medium containing 4% fetal bovine serum. The cells were washed once with Tris-buffered saline and once with medium lacking leucine and valine. Cells were labelled for 30 min at 37 °C with 200 μCi each of ^{3}H-leucine and ^{3}H-valine (Amersham-Searle) in 1 ml of medium containing 4% dialysed calf serum but lacking leucine and valine. The labelled cells were washed twice in ice-cold Tris-buffered saline, scraped off the plate in Tris-buffered saline containing 10 mM DTT and 250 μg ml^{-1} PMSF (added fresh) (1 ml per 100 cm plate)[31] and then sonicated for 2 min in a water-cooled Heat Systems sonicator cup horn (maximum setting). Extracts were made 0.1% in NP40 and 0.05% in deoxycholate, incubated on ice for 10 min, centrifuged at 18,000 r.p.m. for 30 min, and the supernatants stored in aliquots at −80 °C. Aliquots of each extract were reacted with specific antisera or antibody proteins according to the method of Padgett *et al.*[32]. Extracts of approximately 2.5×10^6 cells were preincubated with 50 μl of a 10% suspension of heat-killed formalin-fixed *Staphylococcus aureus* Cowen I bacteria[33] in 25 mM Tris, *p*H 7.4, 10 mM EDTA, 0.35 M NaCl, and 0.15% Triton X-100 (TENT buffer) for 15 min on ice. After removal of the bacteria by centrifugation, purified rabbit β-globin (1 μg) and an excess of either purified goat anti-rabbit β-globin immunoglobulin (provided by S. Boyer, Johns Hopkins), normal goat serum (Microbiological Associates), or rabbit anti-VP1 serum (provided by J. Reiser, Stanford) was added and the extracts incubated overnight at 4 °C. Then 50 μl of *S. aureus* suspension (as described above) were added, the mixtures were incubated for 10 min at 4 °C, and centrifuged for 2 min at 8,000 r.p.m The bacterial pellet was washed four times with TENT buffer and the immune complexes eluted from the bacteria for 1 h in a solution containing 10 mM Tris *p*H 7.4, 1% SDS, and 8 M urea. Electrophoresis of cell extracts or of immune complexes prepared from those extracts was for 3 h at 125 V in a 15–20% gradient acrylamide–SDS gel prepared according to the method of Laemmli[34]. The gel was stained and fluorographed[35] (3-day exposure shown). Tracks a–d are untreated cell extracts; e–h are immune complexes from extracts reacted with goat-anti-rabbit β-globin immunoglobulin; a,e, uninfected cell extract; b,f, SV40-infected cell extract; c,g, SVGT5-βG10A-infected cell extract; d,h, SVGT5-βG12A-infected cell extract. Tracks i,j: immune complexes prepared from SVGT5-βG10A (i) or SVGT5-βG12A (j) infected cell extract reacted with normal goat serum; k, immune complex prepared from SVGT5-βG10A-infected cell extract reacted with rabbit anti-VP1 serum.

inserted a *Drosophila melanogaster* (Dm) DNA segment containing the gene for histone H2B into SV40 DNA (between map positions 0.825 and 0.145) and observed the formation of both a cytoplasmic, polyadenylated SV40-Dm H2B hybrid mRNA and H2B protein during multiplication of the recombinant in monkey cells. Experiments are in progress to examine the expression of a variety of prokaryotic, lower eukaryotic and mammalian genes in mammalian cells using this approach.

SVGT5-RaβG may prove invaluable for testing the effects of mutational alterations on the regulation of SV40 transcription. Deletions could be introduced into those portions of the vector DNA that are implicated in viral mRNA formation and their consequences determined, even in the presence of helper genomes, by monitoring β-globin mRNA and protein synthesis. This approach will be particularly advantageous where the mutational changes either prevent or have no effect on the transcription of particular messages. More generally, this system may be used in conjunction with new methods for site-directed mutagenesis[38,39] to 'synthesise' altered eukaryotic mRNA structures at the DNA level, and to examine their function *in vivo*.

SVGT5-RaβG should be able to transform a variety of mammalian cells, such as mouse, rat and human. Since integrated SV40 genomes do not express the late region, SVGT5-βG transformed cells are not likely to make β-globin mRNAs or β-globin. However, other vectors, which permit transcription of the exogenous gene under early control are being explored for their ability to promote expression of the integrated genes.

β-Globin made during infection of CV1 cells by SVGT5-βG is not stable; following a 30-min labelling period most of the newly synthesised β-globin disappears during a 60 min chase (data not included). Several explanations come to mind: the newly synthesised polypeptide is an altered form of β-globin; factors in the monkey cell cultures actively promote breakdown of β-globin chains; cultured monkey kidney cells lack factors normally present in erythroid cells that stabilise β-globin. Our agenda includes experiments to characterise further the structure of rabbit β-globin synthesised in monkey cells and to determine if the β-globin can be stabilised, perhaps by the addition of haemin to the culture medium or the concomitant production of rabbit α-globin by co-infection with a SVGTαG recombinant genome.

We thank Dr T. Maniatis for providing the *E. coli* culture harbouring the β-globin recombinant plasmid PβG1. All experiments involving the construction and propagation of recombinant genomes were performed according to the stipulations in the existing NIH guidelines: P2 for PβG1 and pBR322-βG and P3 for SVGT5-βG. This research was supported by research grants NIH GM 13235-13 and NCI CA 15513-05. Richard C. Mulligan is a NSF Fellow and Bruce H. Howard is an Investigator in the USPHS.

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letters

Interstellar reddening and distance of Nova Cygni 1978

THE interstellar feature of KI(λ7699 Å) has been observed in the direction of Nova Cygni 1978 with an equivalent width of $W_\lambda \simeq 116$ mÅ and has been used here to deduce a colour excess of $E(B-V) = 0.38 \pm 0.08$ mag. The distance–reddening law to the nova is derived from a sample of stars in the nearby field surrounding the nova. The nova seems to be at a distance of $r \simeq 3.3 \pm 0.6$ kpc with a corresponding absolute visual magnitude at maximum light of $M_{V_0} \simeq -7.5 \pm 0.5$ mag. Nova Cygni 1978 is classified as a fast galactic nova on the basis of its absolute magnitude at maximum light and the rate of decline towards minimum light.

A composite light curve for Nova Cygni 1978 is shown in Fig. 1; the data are a mixture of visual, photographic (prediscovery) and photoelectric measurements[1–3]. A polynomial fit to the data yields a time of maximum light of $t_m = 12.20 \pm 0.04$ September 1978 (UT) when the nova attained a brightness of $m_v \simeq 6.2 \pm 0.1$ mag. Near maximum light and thereafter, high resolution spectra (220 mÅ) were obtained of the nova using the 2.7-m telescope of the McDonald Observatory with a self-scanned Reticon 1024B detector[4] and the coudé spectrograph.

In addition to monitoring various emission features, including Hα, Hβ, OI(λ8446 Å), and HeI(λ10830 Å), spectra were also obtained of the NaI(λ5890 Å) and KI(λ7699 Å) interstellar lines.

The interstellar NaI lines are very strong, having equivalent widths ~550 mÅ; NaI lines arising from the nova are also seen displaced by 626 km s^{-1} to the blue. The KI line, shown in Fig. 2, is weaker and seems to contain two unresolved components. From the measured equivalent width of the KI line ($W_\lambda \simeq 116$ mÅ) a column density of $N(\mathrm{KI}) \simeq 9.3 \times 10^{11}$ cm^{-2} is

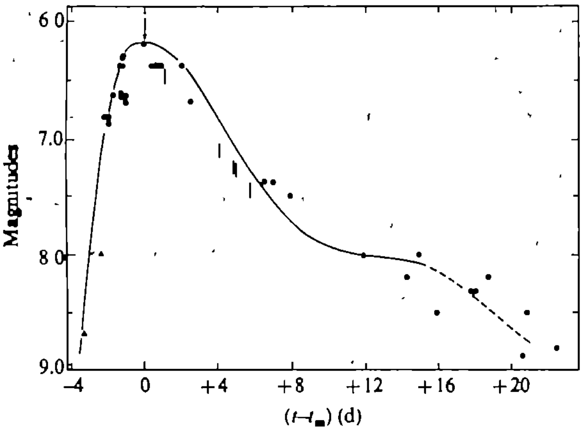

Fig. 1 Composite light curve for Nova Cygni 1978. ▲, Prediscovery photographic values; ●, photoelectric observations; ○, visual observations. Time of maximum light, t_m, (arrow) and the epochs of the spectroscopic data (vertical lines) are indicated. Solid curve approximates behaviour of nova during rise to maximum and early decline.

deduced. This column density can be converted in the $E(B-V)$ colour excess using the mean relationship[5]

$$N(KI) = 6.4\times10^{12}\cdot E(B-V)^2$$

which yields a value of $E(B-V)\simeq0.38\pm0.08$. A higher value of $E(B-V)\simeq0.6$ has been reported[6] from measurements made of both the interstellar K and D_2 lines. Recently, a lower limit of $E(B-V)=0.18$ was determined from linear polarisation measurements[2]; a value of $E(B-V)=0.3$ $(+0.2/-0.1)$ was also reported[1] from an observation of the diffuse interstellar feature at λ6614.

The data given in Table 1 are tabulated for 16 stars[7] in the field near the nova and are used to derive the distance–reddening relationship shown in Fig. 3. The solid line is representative of the general trend. The exact form of the relationship becomes increasingly uncertain beyond 1 kpc due to the paucity of stars with large colour excesses.

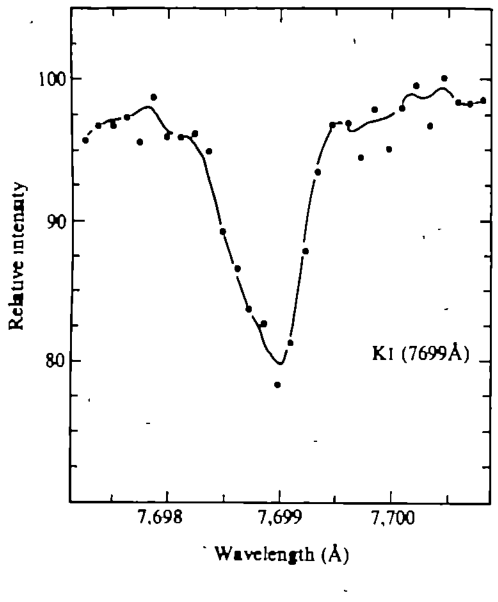

Fig. 2 The KI(λ7699 Å) interstellar feature ($W_\lambda\simeq116$ mÅ) as observed on 16.36 September 1978 (UT). ●, Observed points; Fourier fit indicated by solid line.

Table 1 Reddening in the field of Nova Cygni 1978

Star*	V	M_V	Spectral type†	$E(B-V)$	d(pc)
HD204995	8.66	−1.1	B9(V)	+0.13	751
HD205601	6.76	−1.4	B6 V	+0.08	377
HD206280	6.72	+0.5	B9 V	+0.01	172
HD208513	7.65	+0.2	A0(V)	+0.07	277
HD208835	7.50	−0.1	A0(V)	+0.10	291
HD208878	7.43	−0.1	B8(V)	+0.08	292
HD209469	7.22	−0.2	B9(V)	+0.08	269
HD204153	5.60	+3.0	F0(V)	+0.02	33
HD204536	6.85	−1.8	B3 III	+0.13	461
HD205496	7.99	−0.6	B9(V)	+0.09	454
HD208962	7.60	−1.4	B9(V)	+0.12	544
HD209932	6.44	+0.9	B9 V	+0.04	121
HD209993	6.16	+1.2	A3 V	+0.06	90
HD204710	6.95	−5.6	B8 Ib	+0.33	3,236
HD204722	7.66	−2.5	B2 V	+0.24	1,076
BD+47°3588	9.61	−2.5	B1.5 V	+0.35	2,642

* Ref. 7.

† Luminosity class in parentheses assumed.

A values of $E(B-V)=0.38$ indicates that the nova is located at a distance of $r\simeq3.3\pm0.6$ kpc where the error is estimated from the uncertainty in the distance–reddening law. The visual absorption corresponding to this colour excess is found to be $A_v=R\cdot E(B-V)\simeq1.1$ mag (assuming $R=3.1$) and indicates a value of the corrected apparent visual magnitude at maximum light to be $V_0=m_v-A_v\simeq5.1$ mag. Combined with the above estimate for the distance, this implies a value of $M_{V_0}\simeq-7.5\pm0.5$ mag was reached by the nova at maximum light.

Since the time of maximum, the nova has declined at a rate of ~0.13–0.17 mag d^{-1}. Using the speed classes defined by McLaughlin[8], Nova Cygni 1978 appears as a fast galactic nova, comparable to other fast novae such as EI Aql ($M_V\sim-7.35$) and DI Lac ($M_V\sim-7.3$). Assuming a value $B\sim20$ for the nova precursor[9] implies an absolute magnitude for the precursor of $M_B\sim7$ mag.

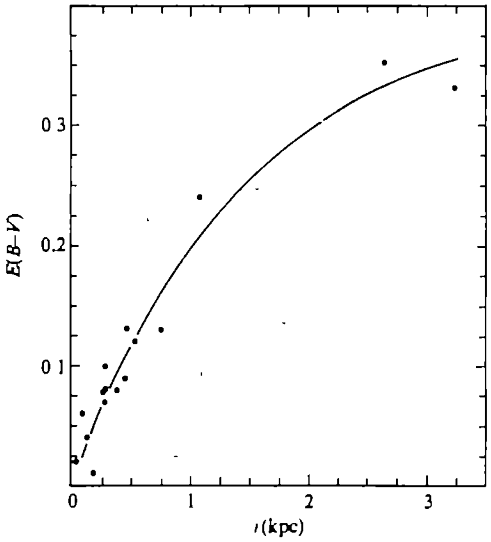

Fig. 3 The distance–reddening law in the direction of the nova. The solid points represent the data from Table 1; the line indicates the form of the reddening curve which is only approximately represented beyond 1 kpc.

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Is Crab nebula a disrupted helium star?

WE propose here that the Crab nebula was produced by the explosion of a helium star in a close binary already containing a neutron star. This explosion would be caused by a complete spiralling of the neutron star into the helium star, which was totally disrupted[1]. The neutron star would be spun up in the process and be identified with PSR0532.

Arnett has pointed out that the overabundance of helium with respect to hydrogen in the Crab filaments[2] is compatible with a presupernova helium core of mass 4 $M_{\odot}$ (ref. 3) which corresponds to a main sequence mass of 12 $M_{\odot}$ (ref. 4). Chevalier[5], on examining old Chinese records, present day nebula and the embedded pulsar, finds that all available information is consistent with the Crab having a massive progenitor. The fact that the motion of the Crab pulsar is directed away from the I Gem association has led to the suggestion[6] that the progenitor of the Crab was a runaway star from I Gem. According to this model, the first supernova explosion in a close binary in I Gem disrupted the binary releasing a neutron star (identified with PSR0527) and a normal star of mass 12 $M_{\odot}$ which later exploded to give the Crab nebula and the pulsar.

The model of a massive progenitor for the Crab as discussed above presents some problems. First, detailed work on the evolution of close binaries in the context of compact X-ray sources has shown (see ref. 1) that the first supernova explosion in a mass transfer binary is unlikely to disrupt the system. (If the binary is sufficiently wide so that mass transfer does not take place, the velocity of the normal star at the time of the disruption of the binary—equal to its orbital velocity—would be very small). Thus we are faced with the problem of placing a massive progenitor for Crab at $|z| = 200$ pc. Second, to avoid an observable overabundance of heavy elements in the Crab filaments, it must be assumed[3] that the explosion of the helium star leaves behind a 1.45 $M_{\odot}$ neutron star core. Now this value is substantially larger than 0.5 $M_{\odot}$ estimated for the Crab pulsar on the basis of detailed models of neutron stars and data on micro- and macroglitches[7]. Third, there is the crucial problem of the mass. For the nebula a total mass of ~1 $M_{\odot}$ has been estimated from the absolute intensities of emission lines[8]. On the other hand, Davidson *et al.*[9] conclude that a nebular mass in the range 0.3–10 $M_{\odot}$ would not be inconsistent with observations, most of the mass being in dense neutral cores of filaments. However, the fact that bright and faint filaments have been accelerated equally, argues against the presence of too much neutral matter[10]. The mass of the nebula is therefore unlikely to be very much greater than 1 $M_{\odot}$. Thus the problem is that if the Crab is a result of the supernova explosion of a massive star, where is the remaining mass?

These problems are solved if we assume that the progenitor of the Crab was a mass transfer binary. It is then obvious that the event of 1054 could not have been the first supernova explosion, because such an explosion would leave the binary intact. In other words the progenitor binary of the Crab must have passed through a Cen X3 type X-ray stage. The evolution of a normal massive star in a close binary containing a neutron star is characterised by a tremendous loss of mass and angular momentum[11–13]. The normal star expands and engulfs the neutron star, which leads to a rapid shrinkage of the orbit[14]. The spiralling in of the neutron star terminates when only the helium core of the companion is left, the hydrogen rich envelope having been blown off[13]. For example[15], starting with an X-ray binary consisting of a 1 $M_{\odot}$ neutron star and a 15 $M_{\odot}$ normal star, we may arrive at a system consisting of a 1 $M_{\odot}$ neutron star and a 3 $M_{\odot}$ helium star and having a period of ~1.5 h corresponding to a separation of about 1 $R_{\odot}$.

It has implicitly been assumed[16,17] that the helium star now evolves as a single star, so that its explosion in most cases distrupts the binary releasing two neutron stars, the old spun up neutron star manifesting itself as a type L pulsar and the newborn as a type D pulsar. However, conditions may be such that the helium star does not evolve as an isolated star. A 3 $M_{\odot}$ helium star has a radius of 1.4 $R_{\odot}$ at the onset of carbon ignition and of 4.5 $R_{\odot}$ at the onset of neon burning[18]. Thus if the separation between the two stars is sufficiently small, at some point in the evolution of the helium star the neutron star would be engulfed, triggering off a new spiralling in, which may totally disrupt the helium star and spin up the neutron star to very short periods[1].

According to our hypothesis, the Crab nebula is such a disrupted helium star and the Crab pulsar, a spun up old neutron star (a type L pulsar). This model has many attractive features. It provides a helium star for explosion without an embarassingly large mass for the nebula. It explains the observed overabundance of helium without postulating an excessively heavy neutron star or an unwanted excess of heavy elements. In this context it is relevant that Arnett[3] finds a better agreement between estimated[3] and inferred[2] abundances if interstellar matter is swept up rather than an old envelope (as would be the case if the exploding star were single).

The spun up neutron star in our model would move with a velocity equal to the centre of mass velocity the progenitor binary had acquired at the time of the formation of the neutron star[1]. (In refs 16, 17 it was assumed that a type L pulsar moves in the orbital plane of the progenitor binary). In the light of the above discussion, the fact that the motion of the Crab pulsar is directed away from the I Gem association permits a more plausible explanation (see ref. 6). The progenitor of the Crab was a massive close binary in I Gem. The first supernova explosion did not disrupt the binary, rather imparted the centre of mass a velocity of about 125 km s^{-1}. In about the 3 Myr between the first and the second explosion, the binary covered a distance of ~380 pc—crossing the galactic plane—and appearing at the site of the explosion of 1054. (Note that in the model of Gott *et al.*[6], the Crab pulsar did not acquire any velocity as a result of the supernova explosion).

The earlier suggestion[17] that PSR0532 is a type D pulsar and the associated type L pulsar should still be embedded in Crab nebula appears to be less attractive because of the problem of the abundance of heavy elements discussed above.

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A sensitive search for radio pulses from primordial black holes and distant supernovae

THERE are two mechanisms of current theoretical interest which might generate short (< 1 ms) radio pulses detectable at interstellar or even cosmological distances. In both cases the pulse is generated by the alteration of a magnetic field by an expanding conducting shell. The phenomenon might occur either as the expanding core of a supernova 'combs' the star's intrinsic dipole field[1,2], or as the shell of charged particles emitted in the explosion of a primordial black hole (PBH) excludes the surrounding magnetic field[3]. In the latter case, the resulting radio pulse would, with suitable particle physics[3,4], be much easier to detect than the γ-ray pulses that might also accompany the explosion[5]. The best published upper limit on the frequency of such γ-ray pulses is 4×10^{-2} events $\mathrm{pc}^{-3}\,\mathrm{yr}^{-1}$ (ref. 6). We present here the results of a radio frequency search for isolated pulses, with much higher sensitivity than achieved previously. No definitive pulses have been detected, and the implied limit on PBH explosions is 2×10^{-9} events $\mathrm{pc}^{-3}\,\mathrm{yr}^{-1}$.

The results of several radio pulse searches have already been summarised, both from the point of view of supernova energies[7] and from that of limits on the number of PBH explosions[8]. Table 1 expands a summarising table from ref. 7, and gives the parameters of the most sensitive and most fully documented previous searches. We have attempted to compute the derived parameters as uniformly as possible, with a minimum of assumptions about the nature of the unseen pulses. Figure 1 shows the relative limits on the rate of pulse-producing events provided by the various surveys, plotted as a function of observing frequency.

Most of the published pulse searches have been carried out with simple receiving equipment and antenna apertures of not more than a few square metres. To achieve a greatly improved sensitivity limit, we have re-analysed data from a pulsar survey[9] done at the Arecibo Observatory in 1973–74. At 430 MHz the Arecibo telescope has an effective aperture of over 40,000 m^2, and the pulsar survey made use of a special dispersion-compensating radiometer that permits a relatively broad total bandwidth to be used without losing sensitivity to impulsive signals. Relevant parameters of the Arecibo observations are summarised in Table 2.

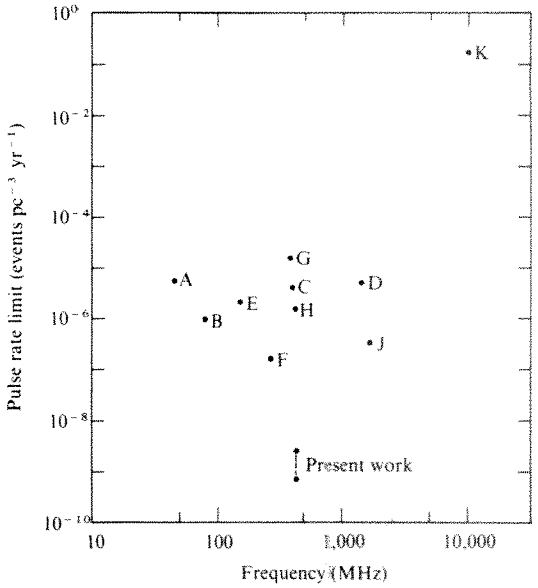

Fig. 1 Approximate limits on the rate of PBH explosions implied by the survey sensitivities listed in Table 1. The lower point plotted for the present work represents the limit as given in Table 1, computed as for the other surveys; the upper point is a firm, 8σ limit based on the data given in Table 2.

Dispersion compensation was done by a small computer that sampled each of the 32 receiver output channels every 16.7 ms and then recombined them with progressive delays to 'de-disperse' the signals. In the observations under discussion the dispersion range was 0–640 cm^{-3} pc (in steps of 20 cm^{-3} pc and with time resolution of 16.7 ms) and 0–1,280 cm^{-3} pc (steps of 40 cm^{-3} pc, time resolution of 33.3 ms). The de-dispersed data were arranged in blocks of 8,192 samples (4,096 for the high-dispersion range), and for each block a two-dimensional histogram was printed showing the distribution of detected, de-dispersed signal amplitudes, as a function of dispersion measure. The observed frequency distributions were indistinguishable from a gaussian curve over the range of amplitudes for which adequate statistics were available (±5 standard deviations of the noise), and were also indistinguishable from those produced when a calibration noise source was inserted into the system.

About 12,000 blocks of data were searched for evidence of narrow pulses of well defined, non-zero dispersion measure.

Table 1 Sensitivity parameters for 11 radio pulse surveys

1 Survey	2 Frequency ν (MHz)	3 Bandwidth $\Delta\nu$ (MHz)	4 Integration time τ (s)	5 System temperature T_s (K)	6 Solid angle Ω (sr)	7 Observing time T (h)	8 Threshold sensitivity $U_{\mathrm{min}}(\mathrm{J\,m^{-2}\,Hz^{-1}})$	9 Maximum distance for PBH d_{max} (pc)	10 Rate of PBH events R ($\mathrm{pc^{-3}\,yr^{-1}}$)
A[11,7]	45	1	0.6	25,000	1	1,768	1.1×10^{-21}	140	$5+10^{-6}$
B[12,7]	80	1	0.6	5,000	1	1,100	1.2×10^{-22}	280	1×10^{-6}
C[13,7]	400	0.06	1.7×10^{-5}	200	3	100	1.4×10^{-22}	270	4×10^{-6}
D[13,7]	1,400	0.5	4×10^{-6}	150	0.25	100	1.4×10^{-23}	580	5×10^{-6}
E[14,7]	151	~1	~1	1,000	~0.8	3,620	3.4×10^{-23}	160	2×10^{-6}
F[16]	270	50×0.25	0.02	200	1	213	1.1×10^{-24}	900	1.6×10^{-7}
G[17]	380	3	0.5	500	0.086	232	4.7×10^{-24}	430	1.6×10^{-5}
H[18]	430	1.5	0.1	1,100	3.3×10^{-4}	33	3.2×10^{-26}	5300	1.6×10^{-6}
J[19]	1665	15	0.002	200	5.6×10^{-5}	28	6.6×10^{-28}	3.7×10^{4}	3×10^{-7}
K[20]	10,000	40	0.5	20,000	1.5×10^{-3}	1,700	6.2×10^{-22}	38	1.9×10^{-1}
Present work	430	2×32×0.25	0.017	175	6.6×10^{-6}	292	1.3×10^{-29}	2.6×10^{5}	7×10^{-10}

Data given in columns 2–7 were taken directly (or estimated) from the original references or from Meikle and Colgate[7]. The 6σ threshold sensitivities (column 8) were computed from the relationship $U_{\mathrm{min}}=6(2kT_s)\Delta tA^{-1}(\tau\Delta\nu)^{-1/2}$ where k is Boltzmann's constant, $A=\lambda^2/\Omega$ is the effective antenna aperture, Δt is the apparent pulse width expected at the receiver output, and the remaining quantities are defined in the table headings. For surveys A–D, Δt will be dominated by dispersion broadening for interesting dispersion measures; the quoted value of U_{min} assumes $\Delta t=8{,}280\,DM\,\Delta\nu\,\nu^{-3}$ for ν, $\Delta\nu$ in MHz and $DM=100\ \mathrm{cm}^{-3}$ pc. For the remaining surveys U_{min} is independent of dispersion measure because Δt is dominated by τ. We have not allowed for differences in antenna efficiencies or the threshold σ values, because few authors gave these. The entries may thus be in error by a factor ~2. Column 9 gives the maximum distance at which a PBH event of $E=10^{16}\ \mathrm{J\,Hz^{-1}}$ could be detected; for surveys A–D we assume a mean density of 0.03 cm^{-3} for the electrons causing dispersion along the line of sight. Finally, column 10 gives the corresponding upper limit on the rate of PBH events. For surveys A–D, $d_{\mathrm{max}}\propto E^{1/3}$ and $R\propto E^{-1}$, while for the others $d_{\mathrm{max}}\propto E^{1/2}$ and $R\propto E^{-3/2}$.

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Blocks with broad, high intensity features at low dispersion measures were rejected as containing terrestrial interference. On all other blocks, all 'events' at or above 6σ were catalogued. The frequency of pulses at the 6σ level was consistent with that expected from random noise fluctuations.

No pulses were found at, or above, the 8σ level, but eight unusual features seen at about 7 times the r.m.s. noise should be mentioned. These events do not seem to be mere statistical fluctuations, and most were found at such high aparent dispersion measures and so isolated that a plausible source of interference is not apparent. However, as they were evenly distributed in dispersion measure, and none appeared at or above 8 times the r.m.s. noise, it seems unlikely that many of them could have been produced by isolated events spaced uniformly over the region surveyed. They are not due to normal pulsars, because the original data was examined for periodicity ($0.033 < P < 3.9$ s) by a computer program which could detect pulsars well below 6 times the r.m.s. noise, and some of them are clearly due to single events that lasted less than the integration time. The pulses might be due to irregular giant bursts from very faint pulsars, or to some completely unknown terrestrial or extraterrestrial cause. A decision on the source would require either vast amounts of observing time with a single Arecibo-sized antenna, or a shorter period with two Arecibo-sized telescopes operating simultaneously.

The sensitivities and pulse rates quoted in Table 1 are based on a 6σ detection threshold and on the assumption that the pulses being sought are shorter than the integration times of the surveys. This assumption seems valid as the pulses produced by the mechanism mentioned earlier are expected to be intrinsically shorter than 10^{-6} s, and broadening of the pulses by interstellar scintillation, if it is like that observed for pulsars[10], should not be a problem, at least out to $DM \approx 500\ \text{cm}^{-3}$ pc (the largest dispersion yet observed for pulsars).

To make a rough estimate of the upper limit on the rate of PBH explosions implied by these observations, we assume that every explosion releases nearly mc^2 of energy over a bandwidth of 1 GHz. With the assumptions introduced by Rees[3] and Blandford[4], and taking the mass of still-unexploded black holes to be $m_{\text{crit}} \approx 10^8$ kg, we find that the explosion releases energy density $E \approx 10^{16}\ \text{J Hz}^{-1}$ at frequencies near $\nu_c \approx 1$ GHz. If the detection threshold is U_{min}, such an outburst could be detected to a distance d_{max} given by

$$U_{\text{min}} = E/(4\pi d_{\text{max}}^2). \tag{1}$$

The volume V surveyed is then

$$V = 1/3\, d_{\text{max}}^3 \Omega \tag{2}$$

where Ω is, say, the solid angle of the area of sky surveyed to better than −3dB of the on-centre sensitivity. The limit on the rate is then taken to be $R = (VT)^{-1}$, these rates are plotted in Fig. 1. The reader is invited to multiply them by the factor appropriate to his favourite distribution and confidence level. Corrections for $E \neq 10^{16}\ \text{J Hz}^{-1}$ may also be applied.

One must be careful in comparing the rates thus obtained, as there are many hidden assumptions (besides the one that the particle physics is such that the phenomenon can occur at all): first, the spectrum of the hypothetical pulses has been asumed flat from over the frequency range covered by these surveys; this is not likely to be the case[4], but because the value of ν_c is uncertain by several orders of magnitude, it is not possible to make any useful corrections.

Second, the magnetic field strength at the sites of the explosions is assumed to be a few microgauss. This is likely to be an overestimate for survey J and the present work, where much of the volume searched was intergalactic. Again, useful corrections are not possible, because ν_c and the strength of the intergalactic field are unknown.

Finally, the PBH's are assumed to be uniformly distributed throughout space. If they are assumed to be correlated with galaxies (into which they might have fallen), or anticorrelated (they might rapidly accrete to masses $>m_{\text{crit}}$ inside galaxies) comparisons become much more difficult.

Table 2 Parameters of the Arecibo search

Frequency	430 MHz
Bandwidth	32×0.25 MHz
Polarisations	2 circular, summed
Integration time	0.0167 s
Effective aperture	$4 \times 10^4\ \text{m}^2$
System noise temperature	175° K
Regions searched	Aquila, Taurus
Interference-free search time*	292 h*
Threshold sensitivity* (8σ)	$3 \times 10^{-29}\ \text{J m}^{-2}\ \text{Hz}^{-1}$

* Values quoted are for the lower dispersion-measure range. The higher *DM* data blocks had threshold sensitivity $4 \times 10^{-29}\ \text{J m}^{-2}\ \text{Hz}^{-1}$ and were interference-free for 368 h.

Any further interpretation of the data in Tables 1 and 2 must await theoretical developments.

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A wind driven shell model for the absorption line systems in QSOs

THE spectra of many QSOs are rich in narrow absorption lines. The still somewhat uncertain identifications suggest that the absorption is due to ions of ionisation potential <40 eV. If the ionisation is due to the radiation from a central source, then theoretical models[1–4] give gas temperatures of <~30,000 K. The absorption line systems are generally blueshifted with respect to the emission line redshift of the object. If these blueshifts are Doppler shifts then they correspond to velocities ranging from ~0.001 to 0.5 of c (the velocity of light)[5]. The interpretation of these differential redshifts has been controversial[5]. Opinion has polarised about two extreme hypotheses—that all the absorption is due to intervening galaxies (the intervening hypothesis) or that it all arises in the vicinity of the QSO (the intrinsic hypothesis). At present, apparently the only way of mapping the Universe at large distances is by observing QSOs. The weakness of current models for intrinsic absorption has lead many to suppose that all the absorption occurs in intervening galaxies. If true, this has profound implications for the structure of the Universe. In particular, it implies that in the early Universe, galaxies had extremely large haloes (perhaps as large as 0.5 Mpc (ref. 6). Furthermore, it suggests that there also existed, at high z, large clusters of galaxies with velocity dispersions much greater than those hitherto observed at small z

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(refs 5, 7, 8). Because of the importance of these conclusions for galactic structure and evolution, it is obviously essential to determine what proportion, in fact, of the absorption systems are intrinsic to the QSOs. We present here an intrinsic model which seems to explain the essential features of many absorption systems. This is a crucial first step towards the eventual separation of the observations into intrinsic and intervening systems.

The parameters given directly by the observations are the column density, ionisation state, velocity and velocity dispersion. It is also possible to estimate indirectly the density of the absorbing material[9] and its distance from the central radiation source[9–11]. Although uncertain[12], these estimates suggest that the absorption takes place in thin, dense ($\sim$10–10^3 cm^{-3}) shells at distances of the order of kpc (ref. 5).

All previous attempts[13–15] to explain these systems as intrinsic have relied on expulsion of gas from a central object. The principal difficulty with such models is that they are unable to produce enough absorbing material at large enough distances from the source. They also cannot account for the apparently normal abundances observed.

It is now generally believed that QSOs are active nuclei of galaxies and it is therefore reasonable to suppose that the absorbing material consists of interstellar gas which has been swept up by winds emanating from QSOs. Such a model gains added credibility from detailed investigations of the physics of radiatively accelerated winds[15,16] which show that it is inevitable that a significant fraction of the radiative energy output of a QSO is converted into kinetic energy of an outflowing wind[16].

If a QSO emits a wind with velocity V, and with a mass-loss rate $\dot{m}$, into a uniform stationary gas of density ρ_0, then the flow will be as shown in Fig. 1. This flow pattern is set up because V is much greater than the sound speed in the ambient gas. The validity of the hydrodynamic approximation is discussed elsewhere[17]. This situation is entirely analogous to the interaction of a high speed stellar wind with interstellar gas[18,19]. In the initial stages of the flow both the shocked ambient and wind material are far too hot either to cool or to give rise to absorption lines. In fact it can be demonstrated that the shocked QSO wind material behaves adiabatically over all time-scales of interest[17]. The effects of radiation pressure on the dynamics of the system can also be shown to be negligible during this phase[17]. During this completely adiabatic phase the position, r_s, of the outer shock can be described by the following similarity law:

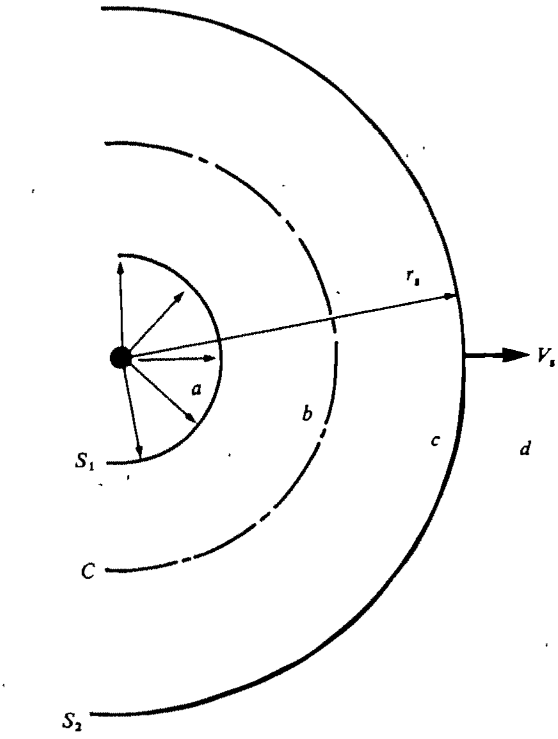

Fig. 1 Flow pattern of a QSO: *a*, the freely outflowing wind; *b*, the shocked wind; *c*, swept up ambient gas; *d*, undisturbed ambient gas. S_1 is an inwards facing shock in the wind, C is a contact discontinuity and S_2 is the outwards moving shock in the ambient gas.

$$r_s = \alpha \left[\frac{1}{2} \frac{\dot{m} V^2}{\rho_0} \right]^{1/5} t^{3/5} \qquad (1)$$

where α is a dimensionless constant which is found from computations[19] to be equal to 0.88. A similarity solution is valid because the characteristic length, $l = (\dot{m}/(V\rho_0))^{1/2}$, is small compared with the typical scale size of the flow for all reasonable values of the parameters. For the swept-up material to be visible in absorption, it must cool. Cooling sets in suddenly[19] when the radiative cooling rate exceeds the expansion cooling rate immediately behind the outer shock (S_2). This occurs at a time t_c when the shock has a radius r_c and velocity V_c given by[17]:

$$t_c \simeq 4.3 \times 10^6 \dot{E}_{46}^{3/11} n_0^{-8/11} \quad \text{yr} \qquad (2)$$

$$r_c \simeq 7.7 \dot{E}_{46}^{4/11} n_0^{-7/11} \quad \text{kpc} \qquad (3)$$

$$V_c \simeq 3.6 \times 10^{-3} \dot{E}_{46}^{1/11} n_0^{1/11} c \qquad (4)$$

In these equations the mechanical energy output rate, $\dot{E} = \frac{1}{2}\dot{m}V^2$ of the QSO is expressed in units of 10^{46} erg s^{-1}. In deriving these equations, the cooling rate has been taken to be $L = 1.68 \times 10^4 nT^{-1/2}$ erg g^{-1} s^{-1}. This is a reasonable approximation[20] for the temperature range, $5 \times 10^4 \text{ K} < T < 5 \times 10^7$ K, and is calculated on the assumption of normal solar abundances. This is the appropriate temperature range for the onset of cooling in all circumstances which have been considered[17]. (The above parameters are not, in fact, very sensitive to the numerical coefficient adopted in the cooling rate. If L is multiplied by a factor A, say, it can be shown that $t_c \sim A^{-5/11}$, $V_c \sim A^{2/11}$, and $r_c \sim A^{-3/11}$.)

Rather than attempting to present here a complete description[17] of results applicable to a wide variety of particular objects, we will now concentrate on the absorption system in the QSO 3C191. We do so because this object was not only the first QSO with identified absorption but is to date one of the very few QSOs which possesses an unambiguously identified set of absorption lines[5]. Studies of the spectra[11] place the absorption line system with a relative velocity of 0.0027 c at a distance of $\sim$10 kpc from the source, and estimate a column density of $N_{\text{HI}} \sim 3 \times 10^{19}$ cm^{-2} and a number density $\sim 10^3$ cm^{-3}. The observed visual luminosity, L_v, is 10^{47} erg s^{-1} (assuming $H_0 = 50$ and $q_0 = 0$).

We will suppose that the spectrum is flat ($\alpha \sim 1$) with a drop or cut off at about the He II ionisation limit. Theoretical studies[16] show that a value of $\dot{E} \sim 10^{45}$ erg s^{-1} is consistent with this spectrum and the observed visual luminosity. For n_0 we adopt the value of 1, but note that the results are rather insensitive to the actual choice of parameters. These values give $t_c = 2.3 \times 10^6$ yr, $r_c \simeq 3.3$ kpc, $V_c \simeq 0.0029\, c$. At this shock speed, the postshock temperature is $\sim 10^7$ K. The gas will now cool according to the cooling law above until it reaches a state of ionisation and thermal equilibrium with the radiation field. The equation of state is now[15,21]

$$P = R\rho y f(y) \qquad (5)$$

where

$$y = L_v/(4\pi r^2 \rho c^3) \qquad (6)$$

is the ionisation parameter[14,21], R is the gas constant, and $f(y) = T(y)/(\mu y)$. This state is critically dependent on the spectrum of the QSO[21]. For flat spectra $F(\nu) \sim \nu^{-\alpha}$ (with $\alpha \sim 1$) the equilibrium temperature is a few times 10^4 K. Calculations[21] of the photoionisation equilibrium show that over many orders of magnitude in y for most spectra, the gas is quasi-isothermal at either ~10^6 K (high ionisation) or ~10^4 K (medium ionisation to neutral). In these quasi-isothermal states a good approximation[15,21] for $f(y)$ is $f(y) \sim By^{-\beta}$, with $\beta \sim 1$, $B(10^6\text{ K}) \sim 6\times10^5$, $B(10^4\text{ K}) \sim 6\times10^3$. As the gas cools V will initially decrease, and an upper limit to the postshock pressure is

$$P_s = \frac{2}{\gamma+1}\rho_0 V_o^2 \tag{7}$$

Then the number density in the cool material is

$$n \leq n_0 \frac{V_o^2}{Ry_o f(y_o)} \tag{8}$$

where y_o is the equilibrium value of the ionisation parameter. Substituting equations (6) and (2) into equation (8), we obtain the implicit equation for y_o:

$$f(y_o) \leq 9.3\times10^{15}\, n_0^{-1/11} \dot{E}_{46}^{10/11} L_{\nu,46}^{-1} \tag{9}$$

$(L_{\nu,46} = L_\nu/10^{46})$

For the values of n_0, $\dot{E}$ and L_ν for 3C191, we find that $\log_{10} f(y_o) \simeq 14.1$. From Röser's[21] calculations we find $\log y_o \simeq -9.5$. For this spectrum, at y_o C IV and Si IV are the dominant ions. During the subsequent evolution[17] y decreases so that the gas becomes more neutral. From y_o and $f(y_o)$ we find that (equation (8)) $n \leq 2.3\times10^3$.

The shell thickness, Δr, is given by

$$\Delta r = \frac{1}{3}\frac{n_0}{n} r_s \tag{10}$$

so that $\Delta r/r \simeq 10^{-4} \ll 1$. (The importance of this small ratio is that it has always been supposed difficult to get such a small ratio in an intrinsic model.) The column density in neutral hydrogen, $N_{\text{HI}} \sim X(1-x)N_{\text{H}}$, where $N_{\text{H}} = n_0 r_o$ and $x = x(y)$ is the degree of ionisation of hydrogen, and X is the mass fraction of hydrogen. At y_o, $(1-x(y_o)) \sim 10^{-4}$, so that $N_{\text{HI}} \simeq 10^{18}\text{ cm}^{-2}$. Since y decreases, N_{HI} will increase.

All these values are in surprisingly good agreement with the observations in view of the crudity of this first model. This agreement can be shown to remain even when we consider non-uniform and non-spherically symmetric ambient density distributions[17].

Once a cool dense shell has formed, it will start to absorb momentum from the radiation field.

We do not discuss the further behaviour of the shell here in detail, because, in contrast with the formation of the shell, the flow depends strongly on the parameters. We show[17] that in the majority of cases, the shell accelerates due to the radiative driving and that often the acceleration is limited only by the total momentum content of the radiation field. If this happens, then it is unavoidable that the shell becomes subject to the radial instabilities discussed elsewhere[22], as well as non-radial instabilities[17]. The more effective the radiation driving, the higher the velocity and the more unstable is the system. Thus the mechanism seems capable of providing a natural explanation for the multiplicity of high velocity systems. In addition, if the shell does accelerate, then there will be some gas which cannot cool, and will be collisionally ionised, which might explain the apparently anomalous appearance of ions of high ionisation potential in otherwise relatively neutral systems.

We have also examined such important questions as the effects of varying the spectral distribution of the radiation field and of departures from spherical symmetry. These considerations place definite constraints on the characteristics of the radiation sources and on the gas distribution in galaxies in order that absorption line systems form by the mechanism discussed above. Note that the removal of gas by the wind will inevitably have important consequences for the further evolution of the galaxies.

Whereas we do not believe that this mechanism can explain all the absorption line systems of QSOs, our detailed exploration of its consequences[17] should provide a basis for distinguishing between intrinsic and intervening systems.

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Measurement of forces between two mica surfaces in aqueous poly(ethylene oxide) solutions

WE have measured the forces between two curved mica surfaces immersed in a solution of 0.01% poly(ethylene oxide) (molecular weight 148,000) in 0.04 M $MgSO_4$ (a good solvent). The experimental technique allows the measureent of (1) forces as a function of surface separation; and (2) the refractive index of the medium between the surfaces[1,2]. The forces exhibited large irreversibilities as well as short-term and long-term time dependent effects, and we conclude that—for observation times less than a few hours—these interactions cannot be described in terms of an equilibrium force law without specifying the previous history of approach of the surfaces.

Figure 1 shows results obtained after 2 h equilibration. The arrows indicate the paths taken in measuring the forces ($A_1 \to B_1 \to C_1 \to A_2 \to \cdots$). Each compression–decompression cycle ($A_n \to B_n \to C_n$) took 5–15 min, during which the surfaces were approached to point B_n and then separated to a large distance, >300 nm, and after a time brought together again ($A_{n+1} \to B_{n+1} \to \cdots$). Three distinct force regimes were distinguished, which we label α, β and γ.

α-Regime: if the surfaces are kept well separated after a decompression for more than 10 min the initial repulsion on approach rises sharply and roughly exponentially, and its onset depends on the closest distance of previous approach (Fig. 1). However, if the surfaces are kept well separated for a few hours the forces always tend to the limiting force law (Fig. 1, dotted line). The repulsive forces in the α-regime extend over 15–30 nm, are reversible over short time periods (a few minutes), and have the same form irrespective of the onset separation. This regime may possibly be associated with the 'diffuse interpenetration' domain discussed by Napper[3]. (We also note that if the surfaces are approached within only 1 or 2 min after a decompression from a small separation the repulsion may be preceded by an attractive region. See Fig. 1, inset.)

β-Regime: on further approach the repulsion increases less rapidly and the force varies roughly linearly with distance, but closer in it rises more rapidly. The β-regime is highly unstable and irreversible: the force depends on the rate of approach, and if the surfaces are kept under a constant force they drift towards each other at 1–5 nm min^{-1}, though closer in the inward drift rate is lower. We have also observed that the surfaces can undergo sudden jumps towards each other, suggestive of the 'yield' characteristic of polymeric gels possessing a network structure undergoing partial collapse[4]. If the externally applied force is decreased in the β-regime the surfaces move out only very gradually or stick, indicating adhesion. The behaviour of the forces in the β-regime may be due to (1) slow adsorption (desorption) processes, including bridging, of tails during compression (decompression); (2) a slow reconformation of a polymeric network undergoing rearrangement of intertail crosslinks. The attractive forces measured during and soon after a decompression from a small separation also strongly suggests that significant bridging occurs.

γ-Regime: below ~5 nm, the repulsion increases very steeply and polymer now becomes forced out from between the surfaces, which ultimately come into molecular contact within ±0.05 nm of the original contact measured in $MgSO_4$ solution without polymer.

We have also measured the refractive index of the aqueous polymer medium between the surfaces at various separations. The refractive index n rises as the separation falls towards the γ-regime, thereby indicating that in the α- and β-regimes polymer is being compressed, though some extrusion may also be occurring. Typical values are: $n = 1.336 \pm 0.003$ at 45 nm, $n = 1.343 \pm 0.003$ at 11 nm. As for bulk polymer solution: $n = 1.334$, and for pure poly(ethylene oxide): $n = 1.454$, these results indicate that the number of adsorbed polymer chains per surface is $\sim 2 \times 10^{11}$ cm^{-2}—a similar value to that obtained for the equilibrium adsorption of high molecular weight poly(ethylene oxide) on silica[5].

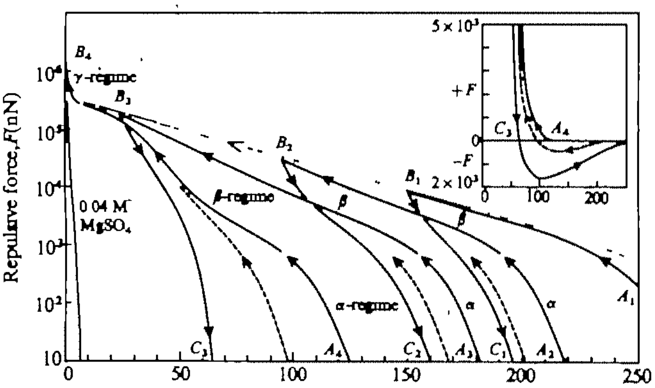

Fig. 1 Repulsive forces $+F$ measured between two crossed cylindrical surfaces of mica of radius ~1 cm as a function of separation D in 0.01% poly(ethylene oxide) in 0.04 M $MgSO_4$ at 21 °C. The force measured before addition of poly(ethylene oxide) is shown on the extreme left and corresponds to pure double-layer repulsion and van der Waals attraction[2,8]. The limiting force with poly(ethylene oxide) (dotted line) is attained a few hours after addition of polymer (for an approach rate of ~10 nm min^{-1}). After compression ($A_n \rightarrow B_n$) and decompression ($B_n \rightarrow C_n$), the subsequent compression forces measured ~1 min later are shown as dashed curves, while those measured 10–20 min later are shifted farther out ($A_{n+1} \rightarrow B_{n+1}$). The inset shows detail on a linear plot of the decompression ($B_3 \rightarrow C_3$) and subsequent compressions ($A_4 \rightarrow B_4$) measured 1 and 15 min, respectively, after decompression C_3.

The magnitude of the forces was different when different micas were used, probably reflecting the different adsorption capacity of poly(ethylene oxide) on different mica surfaces. However, the qualitative features discussed here were always the same, and forces were always measured at distances many times $\langle r^2 \rangle^{1/2}$ (~30 nm) of the poly(ethylene oxide), qualitatively similar to recent results obtained with poly(vinyl acetate)[6,7].

Our results indicate (1) the existence of a dilute long-range polymer layer forming on mica surfaces, probably possessing gel-like properties; (2) that even though the forces may be monotonically repulsive on approach, once significant overlap occurs there is sticking, probably through polymer bridging, resulting in effective adhesion; and (3) as the rate of segmental rearrangement towards equilibrium is very slow (>1 h) the effective forces between colloidal particles with adsorbed olymers of high molecular weight may often be determned by non-equilibrium relaxation mechanisms.

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Recent trends in the $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide

THE $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide has decreased by approximately 0.6‰ over 22 yr according to new direct measurements reported here. Our results offer a way of establishing whether $^{13}C/^{12}C$ ratios of tree rings[1–6] are representative of atmospheric $^{13}CO_2$ variations. We have carried out both isotopic (at Groningen) and concentration (at La Jolla) measurements of atmospheric CO_2 on air samples obtained during 1977 and 1978 at three widely spaced locations: La Jolla, California (33°N, 117°W), Fanning Island (4°N, 159°W) and the South Pole. Sampling, instrumental, and analytical procedures closely matched a similar study carried out 22 yr earlier by Keeling[7,8].

The new isotopic measurements are plotted in Fig. 1 as the per mil variation, $\delta^{13}C$, of the $^{13}C/^{12}C$ ratio from the historic standard PDB. Also plotted are isotopic data for 1955 and 1956 for those sites and times of day of the original study having minimal local disturbance to the CO_2 concentration from vegetation. Published 1955–56 $\delta^{13}C$ values have been recomputed from the original mass spectrometer mass ratios, 45/44 and 46/44, to be consistent with the computational methods used for the 1977–78 data. Corrections for valve mixing and ^{17}O

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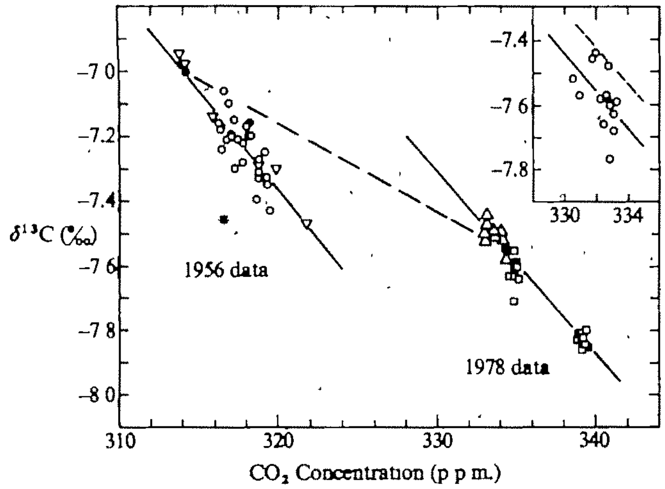

Fig. 1 Change in the relation between carbon isotopic ratio $\delta^{13}C$, and concentration of atmospheric CO$_2$ over 22 yr. Mean change is shown as a dashed line. Locations as follows: ○, Inyo Mountains; *, Inyo sample omitted in computing equation (2), ▽, three west coast United States sites shown for comparison (only samples collected nearest noontime are plotted), Olympic Beach[7], Yosemite National Park[8], Organ Pipe National Monument[8]; △, Fanning Island; □, La Jolla, California. Solid lines show mixing relations for 1956 and 1978 as given by equations (2) and (1), respectively. ●, denote inferred Northern Hemispheric means for 1956 and 1978. Inset: relationship of $\delta^{13}C$ to concentration for the South Pole. Solid line shows mixing relation assuming same slope as Northern Hemisphere (shown dashed.)

follow prescribed methods[9]. The new measurements are calibrated against NBS-20 and a set of organic standards in use since 1963 (ref. 10), whereas the 1955–56 measurements are against PDB itself and two other carbonate standards previously calibrated against PDB. Recomputation has lowered the 1955–56 $\delta^{13}C$ values by 0.09 to 0.10‰. Details of this recomputation, and instrumental and analytical details of the new measurements, will be published elsewhere.

The calibration accuracy of the 1955–56 data is estimated to be within ±0.13‰; that of the new data ±0.05‰.

As seen in Fig. 1, the 1977–78 Northern Hemisphere samples irrespective of latitude closely follow a single mixing equation[7] (in per mil):

$$\delta = -26.54 + 6346.98/\mu \qquad (1)$$

where the first term denotes the $\delta^{13}C$ of CO$_2$ added or subtracted from the sampled air by either fossil fuel combustion or plant activity (which cannot be distinguished); μ is the CO$_2$ concentration in p.p.m. of dry air. We adopt a provisional estimate of the seasonally adjusted concentration for Mauna Loa Observatory (unpublished) as representative of the Northern Hemisphere for 1 January 1978; thus $\mu_{78} = 334.2$ p.p.m. It follows from equation (1) that $\delta_{78} = -7.55$‰. A similar analysis for the Southern Hemisphere (South Pole data) yields $\delta_{78} = -7.59$‰ for $\mu_{78} = 332.6$ p.p.m. Thus no appreciable mean isotopic gradient appears to exist between hemispheres in spite of a small gradient in the seasonally adjusted concentration.

For 1955–56 the Inyo Mountain series (37°N, 118°W) of March 1956, are the most extensive set of data for a single location. These data obey the mixing equation:

$$\delta = -26.97 + 6271.63/\mu \qquad (2)$$

To obtain a representative value of μ we extrapolated backwards from early 1958 when the first precise concentration measurements were obtained at Mauna Loa Observatory[11] on the assumption that the CO$_2$ varied in proportion to the known input from fossil fuel between 1956 and 1978. For January 1, 1956 we find $\mu_{56} = 314.1$ p.p.m. It follows from equation (2) that $\delta_{56} = -7.01$‰.

The above isotopic ratios for both 1956 and 1978 are influenced by atmospheric N$_2$O which is included in the sample gas analysed by the mass spectrometer[12]. To correct for this we assumed a present N$_2$O concentration of 0.30 p.p.m. and a 1.5% increase in N$_2$O per decade[13]. The correction is +0.32‰ in 1956 and +0.31‰ in 1978.

We find that the composition of atmospheric CO$_2$ over the Northern Hemisphere has changed from 1956 to 1978 as follows:

CO$_2$ concentration (μ): 314.1 to 334.2 p.p.m.

carbon-13 ratio (δ): −6.69 to −7.24‰

For the Southern Hemisphere no isotopic data exist for 1956. Concentration data since 1958 (ref. 14) and the present isotopic data indicate, however, that differences between both hemispheres cannot be large compared to hemispheric changes in 22 yr. We therefore regard the Northern Hemisphere data as being close to global averages.

The observed decrease in $\delta^{13}C$ over 22 yr agrees to the stated accuracy with predictions of the effect of fossil fuel combustion on the carbon cycle[15] based on two recently published geochemical models[16,17]. In summary, these models yield almost identical predictions if the land biosphere is assumed to respond to the recent atmospheric CO$_2$ rise[17] such that the observed increase (20.1 p.p.m. or 43 Gton C) is satisfied. When the isotopic fractionation factors (kinetic air to sea, 0.986; equilibrium air to sea, 1.009; air to biosphere, 0.982; air to fossil fuel, 0.980) are treated without mathematical approximation[18], the decrease in $\delta^{13}C$ is 0.44‰ for a fossil fuel CO$_2$ input of 79 Gtons (ref. 17 and unpublished data). This result assumes that the biosphere grew in 22 yr by 12 Gtons. The model predictions, however, are insensitive to biospheric growth which can even be assumed negative. For example, the models predict a change of 0.42‰ if the biosphere shrunk by 40 Gtons, attended by a much greater oceanic uptake of CO$_2$ than assumed in the first calculation (76 instead of 24 Gtons).

This circumstance suggests that a time record of atmospheric ^{13}CO$_2$ data cannot help decide the rate of biospheric growth or shrinkage during this 22 yr period of concentration measurements. Such a record may, nevertheless, be a valuable substitute before the time of reliable direct concentration measurements. Our results are offered as an aid in verifying tree ring studies which span both the past and recent periods.

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Effect of variable energy input on nitrogen fixation in instantaneous linear discharges

ATMOSPHERIC nitrogen oxides are a central issue in the photochemistry of the troposphere, stratosphere and urban atmosphere. The removal of nitrogen oxides by precipitation is a source of fixed nitrogen to the biosphere. Thus the nitrogen fixation rate by natural and anthropogenic discharges is of wide concern[1–8]. While it is generally assumed that P, the molecules of NO produced per joule of discharge, is independent of the input energy, the analysis reported here implies that P does vary with input energy. Thus care must be taken when extrapolating NO yields from small laboratory sparks to more energetic processes of potential geochemical significance. Although the calculations assume a cylindrical symmetry appropriate for a linear discharge, the results are consistent with equivalent calculations using spherical symmetry.

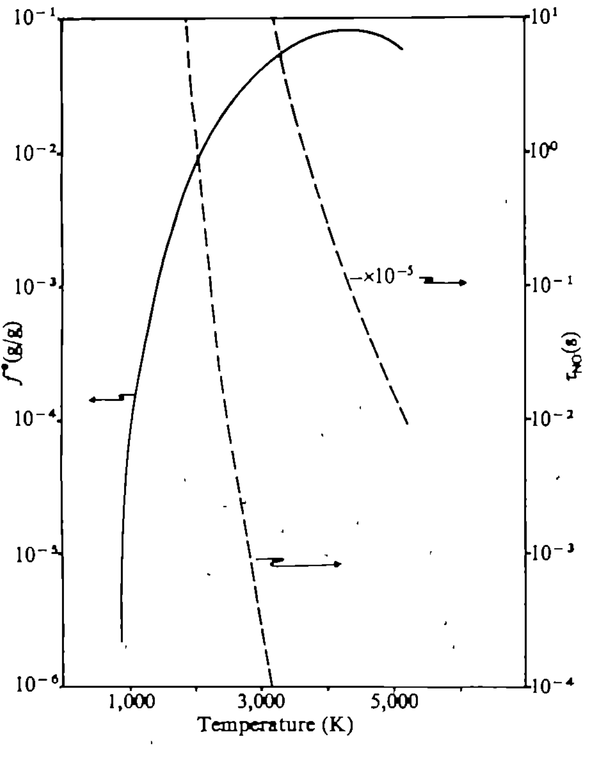

Fig. 1 The NO equilibrium mass mixing ratio, f°, represented by the solid line, and the NO chemical lifetime, τ_{NO}, represented by the dashed line, as a function of temperature, T. Thermochemical data from the JANAF tables[9] was used to determine f° from an initial mixture of 0.78 atm N_2, 0.21 atm O_2, 0.01 atm H_2O, 3.3×10^{-4} atm CO_2, and 1.5×10^{-6} atm CH_4, and ambient temperature of 288 K, and an assumed n_{M}, number density within the heated air, of 2.5×10^{19} molecules cm^{-3}. The equilibrium concentrations as a function of T were determined for N, N_2, NH_x ($x = 1, 2, 3$), NO, NO_2, O, O_2, O_3, H, H_2O, H_2, HCN, OH, HO_2, CO, CO_2, CH_x ($x = 1, 2, 3, 4$), N_2O and HNO. The rates of the reactions $NO + N \rightarrow N_2 + O$, $NO + O \rightarrow N + O_2$, and $NO + NO \rightarrow N_2O + O$ were used to determine τ_{NO} with kinetic data obtained from Baulch *et al.*[10] and Hampson and Garvin[11].

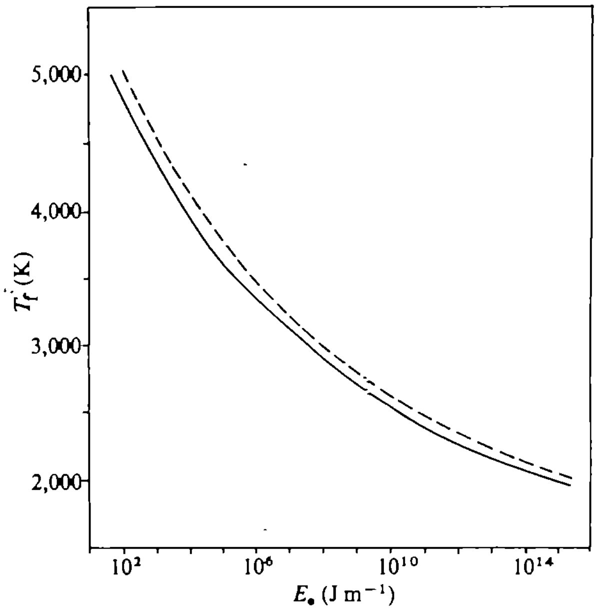

Fig. 2 The freeze-out temperature, T_{F}, as a function of discharge energy, E_0, as determined from equation (8). The solid line is for $\gamma = 1.4$ and the dashed line is for $\gamma = 1.25$.

As identified by Zel'dovich[1,2], N_2 and O_2 are converted to NO by high temperature chemical reactions occurring in the shock-heated air surrounding the discharge. Figure 1 shows f°, the equilibrium NO mass mixing ratio, and τ_{NO}, the NO chemical lifetime, as a function of temperature, T, for an initial mixture of air at 1 atm and 288 K. Defining n_{M} as the number density of the heated air, τ_{NO} is accurately represented by

$$\tau_{\mathrm{NO}} = \frac{1.55\times10^{-2}\exp(53{,}942/T)}{n_{\mathrm{M}}^{1/2}} \qquad (1)$$

(Calculations indicate that for the region of interest n_{M} is 1–10 times the ambient number density. As n_{M} increases, f° slowly decreases.)

If a parcel of air is heated to $T \geq 4{,}000$K and cooled at a rate characterised by a cooling time, $\tau_{\mathrm{T}}(T)$ NO will remain in equilibrium until $T = T_{\mathrm{F}}$, the 'freeze-out' temperature, where

$$\frac{\tau_{\mathrm{T}}(T)}{\tau_{\mathrm{NO}}(T)} \begin{cases} > 1 \text{ for } T > T_{\mathrm{F}} \\ = 1 \text{ for } T = T_{\mathrm{F}} \\ < 1 \text{ for } T < T_{\mathrm{F}} \end{cases} \qquad (2)$$

For $T < T_{\mathrm{F}}$, NO is too long-lived to adjust to the decreasing temperature and NO is frozen at $f^\circ(T_{\mathrm{F}})$. The NO yield is approximated by

$$P = \frac{M(T_{\mathrm{F}}, E_0) f^\circ(T_{\mathrm{F}})}{E_0} \, \frac{6.02\times10^{23}}{30\times10^{-3}} \quad (\text{molecules J}^{-1}) \qquad (3)$$

where M is the mass (kg m^{-1}) of air heated above T_{F} and E_0 is the input energy (J m^{-1}).

To evaluate τ_{T} and M we adopt the numerical-difference equations derived by Lin[12] which simulate a strong shock produced by an instantaneous linear discharge. While these equations do not directly consider translational, rotational, vibrational and chemical modes of energy transfer, by assuming specific heat ratios, γ, of 1.4 and 1.25, these effects should be adequately bounded[13]. For an ambient pressure, p_0, of 1,013

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nsity, ρ_0, of 1.228 kg m^{-3}, T^1, the shock front e, is

$$T^1 = A(\gamma)\frac{E_0^{1/2}}{t} \quad \text{(K)} \tag{4}$$

where t is the time in seconds after the discharge and

$$A(\gamma) = \begin{cases} 1.11 \times 10^{-4} & \text{for } \gamma = 1.4 \\ 6.18 \times 10^{-5} & \text{for } \gamma = 1.25 \end{cases} \tag{5}$$

Following Zel'dovich and Raizer[1], we define τ_T at $T^1 = T_F$ as

$$\tau_T(T_F) = \left(\frac{1}{T^1}\frac{dT^1}{dt}\right)^{-1}\Bigg|_{T^1 = T_F} \tag{6}$$

from equation (4)

$$\tau_T(T_F) = \frac{AE_0^{1/2}}{T_F} \tag{7}$$

Thus τ_T increases as E_0 increases, or the cooling rate decreases as the surface-to-volume ratio decreases.

Combining equations (1), (2) and (7), for $n_M = 2.5 \times 10^{19}$ cm^{-3},

$$E_0 = \left[\frac{3.0957 \times 10^{-12}}{A} T_F \exp(53942/T_F)\right]^2 \quad (\text{J m}^{-1}) \tag{8}$$

Figure 2 illustrates the resulting inverse relationship between T_F and E_0 for $\gamma = 1.4$ and 1.25. While $T_F \sim 2{,}000$ K for $E_0 \sim 10^{14}$–10^{15} J m^{-1}, for lower energies $T_F > 2{,}000$ K. These results contradict previously held assumptions that $T_F \sim 2{,}000$ K, independent of E_0, and, as outlined below, implies that P varies with E_0.

From Lin's[12] equations,

$$M(T_F, E_0) = \pi R^2(T_F, E_0)\rho_0 = \frac{C(\gamma)E_0}{T_F} \quad (\text{kg m}^{-1}) \tag{9}$$

where R is the shock front radius and

$$C(\gamma) = \begin{cases} 3.95 \times 10^{-4} & \text{for } \gamma = 1.4 \\ 1.71 \times 10^{-4} & \text{for } \gamma = 1.25 \end{cases} \tag{10}$$

Substituting in equation (3)

$$P = C(\gamma)\frac{f_0(T_F)}{T_F}\frac{6.02 \times 10^{23}}{30 \times 10^{-3}} \quad (\text{molecules J}^{-1}) \tag{11}$$

The dependence on E_0 is caused by the variation of T_F with E_0. Because $R^2 \propto 1/\rho_0$, P is not directly dependent on ρ_0 or p_0; the variation of τ_T and f^0 with ambient conditions results in only a weak dependence of P on p_0 and ρ_0.

Figure 3 depicts P for $\gamma = 1.4$ and 1.25 as well as some laboratory results[7,14,15], which are consistent with the variations predicted here. The equivalent NO yields for megaton point explosions determined by previous investigators[3–6] which are included in Fig. 3 are also consistent with the present results.

As E_0 decreases from 10^{15} to 10^4 J m^{-1}, f^0/T_F increases and thus P increases. For $E_0 < 10^4$ J m^{-1}, however, $T_F > 4{,}000$ K, f^0 maximises and then decreases, and thus P also decreases. Over the range 10^1–10^{16} J m^{-1}, P varies by a factor of about 9 with a maximum near 10^4 J m^{-1}. Decreasing γ from 1.4 to 1.25, reflecting an increase in heat capacity, causes a 50% decrease in P.

In addition to the above dimensional calculations, fully time-dependent calculations were carried out, similar to those of Goldsmith *et al.*[5], to confirm the behaviour of P. Using Lin's[12] equations, the temperature and density histories of 11 mass shells (of 10^{-5}, 10^{-3}, 2×10^3, and 2×10^7 kg m^{-1} for $E_0 = 10^3$, 10^5, 10^{11}, and 10^{15} J m^{-1}, respectively) extending radially outward were obtained. These parameters as a function of time were coupled to an $N_2/O_2/NO$ chemical scheme[5,10,11] to determine the NO production within each shell. The results of these kinetic calculations are also indicated in Fig. 3. Because the kinetic calculations included the enhanced densities of the shocked air while the dimensional calculations assumed $n_M = 2.5 \times 10^{19}$ cm^{-3}, the yields from the former method are somewhat lower than those from the dimensional approach. The finding from both calculations that P may vary by as much as an order of magnitude over the range of input energies is of potential geochemical significance. An illustrative example is presented below.

To estimate the fixation rate by atmospheric lightning, Chameides *et al.*[7] adopted a P of $(6 \pm 1) \times 10^{16}$ molecules J^{-1} on the basis of 10^1 J m^{-1} spark experiments and simple calculations assuming $T_F \sim 2{,}000$ K. However, if a lightning stroke dissipates about 10^5 J m^{-1} (ref. 16), a value of $(8$–$17) \times 10^{16}$ molecules J^{-1} may be more appropriate, assuming that the highly idealised shock wave model used here adequately simulates the cooling of air around a lightning discharge. Taking 1.6×10^{-7} J cm^{-2} s^{-1} (ref. 7) as the total energy dissipated by lightning, we obtain a global NO production rate of $(1$–$2.5) \times 10^{10}$ NO molecules cm^{-2} s^{-1} or $(35$–$90) \times 10^{12}$ g N yr^{-1}. This may be an overestimation if a large portion of the stroke energy is consumed by the leader or if multiple-stroke flashes do not additively increase the NO yield. For comparison, it is estimated that ~ 20–30×10^{12} g N yr^{-1} are produced by anthropogenic activities[17,18]. As nitrogen oxides are removed from the atmosphere at a rate of $\sim 90 \times 10^{12}$ g N yr^{-1}[7,19,20], it is likely that lightning and combustion are the major sources of atmospheric nitrogen oxides[18,21], both processes involving the conversion of N_2 and O_2 to NO by high temperature chemical reactions.

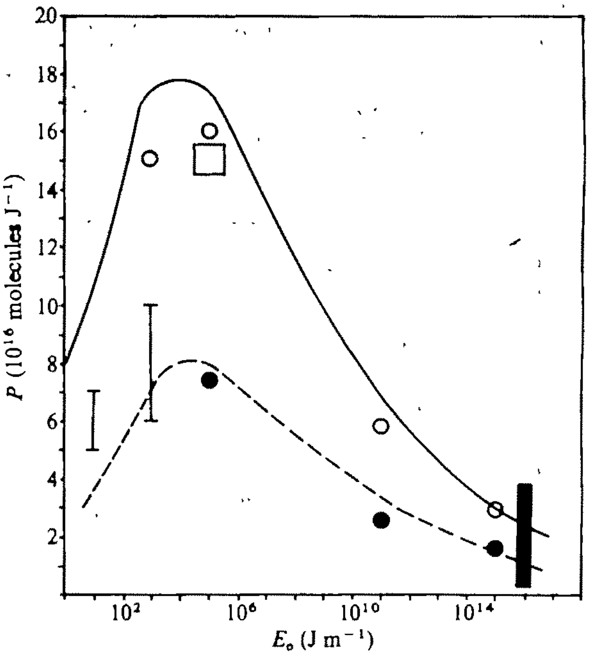

Fig. 3 The yield of NO, P, as a function of discharge energy, E_0. The solid line is for $\gamma = 1.4$ and the dashed line is for $\gamma = 1.25$. The results of the fully time-dependent calculations for $\gamma = 1.4$ (○) and $\gamma = 1.25$ (●), are given. The vertical lines depict the experimental findings of Chameides *et al.*[7] and the square the results of the experiments of Zipf and Dubin[14,15]. The vertical rectangle indicates the equivalent results obtained by previous investigators using spherical geometry for explosions in the megaton range[3–6].

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Black Sea sterol—a molecular fossil for dinoflagellate blooms

A PECULIAR sterol has been found in recent sedimentary environments which we eventually found was identical with the Black Sea sterol. This sterol was first mentioned among the extractable sterols from the Black Sea sapropel layer as the dominant component[1]. The concentration of this sterol in these rare sediments seemed high enough for us to isolate and purify the object to determine its molecular structure and evaluate its significance. We have isolated and analysed the sterol fractions from the Black Sea sapropel (by techniques to be described elsewhere), but here only elucidate the molecular structure of the Black Sea sterol and discuss its significance.

The sediment investigated, which was deposited during the rise of the oxic–anoxic (H_2S) interface after the last glaciation in the Black Sea (7,000–3,000 yr BP), has an organic matter content up to 50% and is composed of numerous membranous structures[2]. During this period the surface water of the Black Sea gradually changed from fresh water to marine conditions. This was accompanied by a changing phytoplankton population from fresh water diatoms and dinoflagellates to marine coccolithophores in the period after 3,000 yr BP (ref. 2). The oceanographic conditions in the brackish water period together with special pathways of organic matter degradation, transformation and preservation in the gradually more anoxic deep water, must have led to the deposition of the unusually organic rich sediment. The abundance of dinoflagellate cysts in the sediment points to an important role of these algae in the brackish water period, which is understandable considering the wide salinity tolerance of dinoflagellates[3]. We feel that the occurrence of the Black Sea sterol could be related to the postulated abundance of dinoflagellates in the surface waters during the period 7,000–3,000 yr BP. The knowledge of the exact structure of this peculiar and abundant sterol in the sediment must give clues to its biosynthetic origin and subsequently to the origin of the sedimentary organic matter. The sediment investigated was sampled by gravity corer in the Western abyssal basin of the Black Sea (41°39′ N, 30°44′ E, −2,150 m). The core (covered core length 118 cm) consisted of a white top layer (0–27 cm), a dark green microlaminated layer (27–71 cm) similar in appearance to the Unit 2 sediment of Ross and Degens[4], and clayish sediments (71–118 cm). The section from 27 to 71 cm was received frozen for analysis. The sediment was homogenised in a Waring blender and divided into portions of 250 g. Total sterols were isolated from the extract of such a portion, obtained after saponification of the wet sediment by ethanolic KOH, followed by digitonin precipitation for isolation of the 3β-sterols. The digitonin precipitation procedure was carried out by addition of an excess amount of a 1% digitonin solution in 90% ethanol to the extract dissolved in C_2H_5OH–CH_2Cl_2 (3 : 1, v/v).

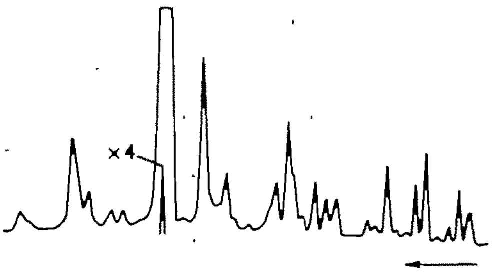

Fig. 1 Capillary gas chromatogram of the total sterols isolated from the Black Sea sapropel layer.

After stirring for two hours at room temperature, the mixture was centrifuged and the precipitate washed thoroughly with ethanol. The precipitate was refluxed in pyridine for 30 min. Digitonin was precipitated from the reaction mixture by addition of diethyl ether. The diethyl ether–pyridine solution was treated with 2 M HCl and the ether layer, containing the freed 3β-sterols, was dried over anhydrous Na_2SO_4. The isolated sterol fraction was transformed into the corresponding trimethylsilylethers by BSA (bistrimethylsilylacetamide, Pierce). Gas chromatographic separation was achieved on a Perkin Elmer 990 instrument, equipped with a 25 m glass capillary column (i.d. 0.25 mm) coated with OV 101. The temperature was programmed from 140 to 280 °C at a rate of 4 °C min^{-1}. Helium was used as carrier gas. Figure 1 shows the appropriate part of the capillary gas–liquid chromatography trace of the sterol fraction. The major peak in this gas chromatogram, previously designated as 'Black Sea sterol', was the subject of a detailed structural analysis. Figure 2 shows the mass spectrum of the Black Sea sterol trimethylsilylether derivative, which was obtained by gas chromatography/mass spectrometry on a Hitachi MS 52 instrument equipped with a 42-m glass SCOT column (i.d. 0.75 mm) coated with OV 101.

The molecular ion *m/e* 500 points to a C_{30} steroidal alcohol with one double bond equivalent. The fragment ions *m/e* 229, *m/e* 231, *m/e* 269 and *m/e* 271 indicate a ring saturated steroid skeleton with one 4-methyl group[5]. The mass spectrum is identical to the mass spectrum of the trimethylsilylether derivative of Dinosterol (4α-methyl-5α(*H*)-Δ^{22}-23,24-dimethyl-cholestan-3β-ol)[6].

Further evidence for the possible similarity between the Black Sea sterol and dinosterol was obtained by comparative studies using ^{1}H NMR and high-resolution gas chromatography.

For that purpose the crude Black Sea sterol was purified further by preparative thin layer chromatography on silica gel with $CHCl_3$–CH_3OH (99 : 1, v/v) as eluent. Crystallisation of the purified sterol yielded 200 μg of needles with a melting point of 220–220.5 °C. The melting point of dinosterol has been reported[7] to be 220–222 °C.

The ^{1}H NMR was measured on a Varian CFT-20 spectrometer in $CDCl_3$. The spectrum of the Black Sea sterol was found to be identical with the one of dinosterol showing a vinyl proton at δ4.96 (1H, q, $J = 1.1$, 10 Hz) and vinylic methyl protons at δ1.59 ($J = 1.1$)[6,7]. High resolution gas chromatography was performed on a Perkin Elmer 990 equipped with glass capillary column on two different polymers (OV-101 and Carbowax high polymer). The retention times of the silylated derivatives of both sterols were identical on co-injection.

These data demonstrate that the Black Sea sterol possesses a structure identical to that of the dinosterol, being 4α-methyl-5α(*H*)-Δ^{22}-23,24-dimethyl-cholesten-3β-ol. As dinosterol has

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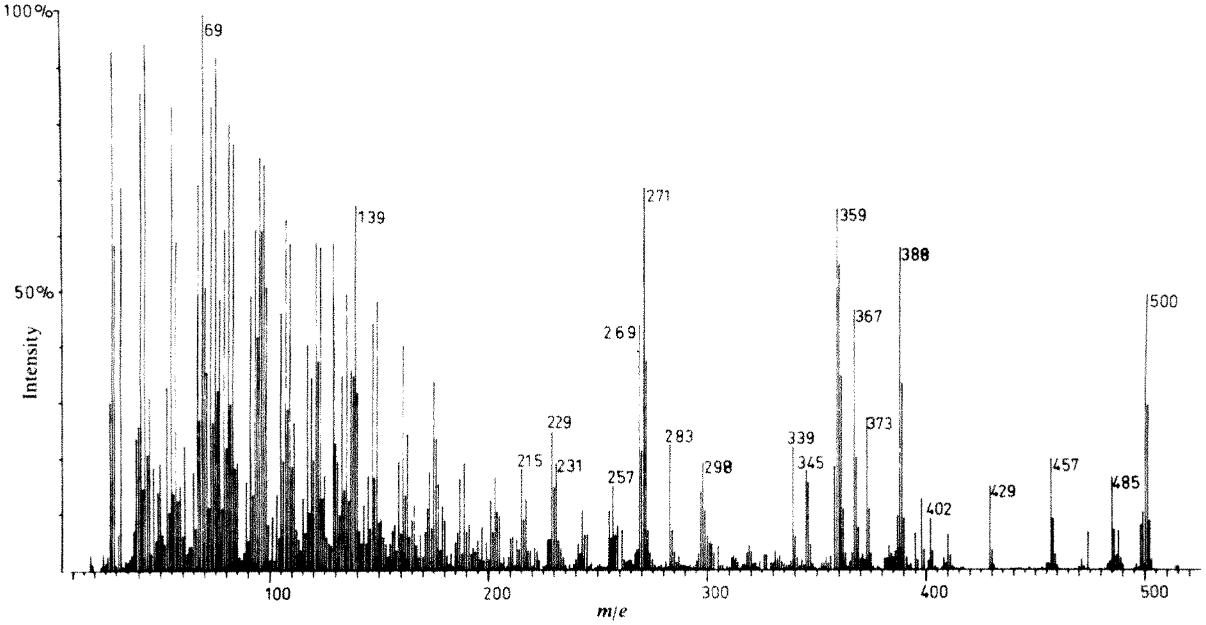
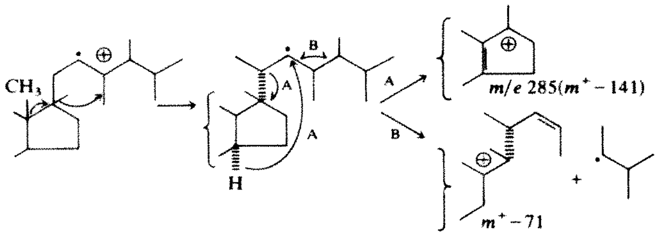


Fig. 2 Mass spectrum of the Black Sea sterol obtained by capillary GC–MS of the silylated sterol fraction. The Δ^{22}-23,24-dimethyl unit in the side chain can be recognised in the mass spectrum by m^{+}-71 and m^{+}-71–90. A fragmentation process is postulated in view of literature data[15]:



previously been encountered as the major sterol in some dinoflagellates only[6], we assume that the abundance of this sterol in the extract of the sapropel layer of the Black Sea sediment reflects a relatively large contribution of dinoflagellate organic matter to this sediment. The remarkable richness in organic matter of the sapropel layer compared with the present marine deposits (Unit 1)[4] suggests periodic blooming of these algae in the brackish water ecosystem as the possible mechanism for organic matter supply, as otherwise similar conditions of preservation and allochthonous sediment influx for both sediment types exist.

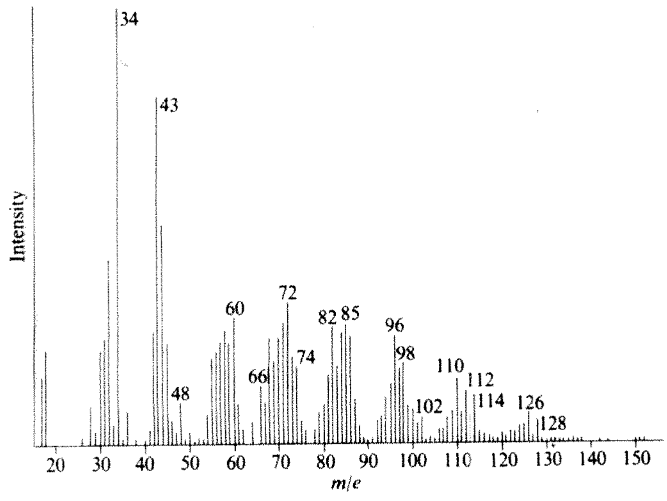


Fig. 3 Curie-point pyrolysis mass spectrum of homogenised Black Sea sapropel. Pyrolysis temperature: 610 °C; ionisation voltage: 14 eV.

To establish the relative contribution of organic matter from different sources, an analysis of the bulk composition of the organic matter by Curie point mass spectrometry was undertaken[8]. This relatively new technique has demonstrated its merits in the analysis of bacteria, fungal cell walls, humic substances and kerogen[9–12].

The pyrolysis mass spectrum of the sapropel (Fig. 3) shows the abundant occurrence of pyrolytic fragments typical of polysaccharides such as *m/e* 32, 60, 72, 74, 82, 85, 96, 98, 102, 110, 112, 114, 126 and 128; and to some extent aminosugar polymers such as *m/e* 95, 97, 109, 125, 137, 151.

Pyrolytic fragments typical of proteins such as *m/e* 17, 34, 48, 67, 92, 94, 108, 117, 131, and lignin such as *m/e* 94, 108, 124, 138, 150, 152, 164 are very low or even absent[10,11].

The pyrolysis fragments *m/e* 17, 34, 36, 38 and 48 are due to compounds such as ammonia, seasalt (HCl) and sulphur compounds (H_2S and CH_3SH). The intense H_2S peak is usually encountered in sediments with active or fossil sulphur-reducing activity.

As the thecal plates of dinoflagellates are mainly composed of polysaccharides[13], we think that the bulk of the Black Sea sapropel is mainly built up from dinoflagellate remains.

Contributions of bacterial organic matter previously based on the analysis of the sedimentary fatty acids[14], is now substantiated by the presence of aminosugar polymers, which make up the cell walls of bacteria.

The Black Sea sapropel unit therefore should be considered as a dinoflagellate ooze, partly reworked by anaerobic bacteria.

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Elimination of natural scrapie in sheep by sire genotype selection

SCRAPIE, a subacute neuromuscular disease of middle-aged sheep, has been attributed variously to an infection or to heredity[1,2]. The clinical disorder follows progressive symmetrical decay of neurones in localised brain sites, notably the hypothalamo–neurophysial and olivo-pontocerebellar 'systems' (ref. 3), associated with a terminal axon dystrophy[4–9]. An artificially transmissible spongiform encephalopathic agent (TSEPA), or 'slow virus', to which many attribute the disease[10,11], is commonly present in diseased animals but its aetiological role remains unclear. Based on the clinical manifestation of natural scrapie, I have proposed that the disease is controlled by an autosomal recessive gene[1,2,12]. This hypothesis allowed identification of the three presumptive genotypes (*ss*, *sS*, *SS*), and precise prediction of attack rates[13,14], amenable to quantitative experimental verification[15]. The evidence summarised here, which has been compiled since 1958, provides strong support for my hypothesis.

The data have been collected from stud sheep flocks, each of 50–300 ewes, of 11 breeds in England and Scotland[12,16]; a 25-yr continuous record of one breeder group of 15–20 flocks with 2,500 ewes provides the principal data, which are supplemented by records of my own experimental flock of 250. Only 35–40% of the females and 1–2% of the males in the flocks have been kept past the age of 1½ yr. Half of these survive to 4½ yr and a few to 10 yr. Ten other breeder groups with 200 flocks have provided data over shorter periods, to give records on some 40,000 individuals.

The diagnosis of scrapie was based on (1) clinical signs[2], (2) the family and clan health history[2], (3) autopsy after euthanasia[3], (4) 'blind' histopathological assessment by light microscopy[3,12] with occasional use of electron microscopy[4–6] and immunohistochemistry[7–9] and (5) occasional assay for the TSEPA. Of about 3,000 affected animals, I have observed 2,100 and autopsied 1,900, of which 1,040 were examined histopathologically, with only two disagreements with the other criteria.

As, in our population, 85–90% of all cases of scrapie were recognisable clinically by 4½ yr of age[2], I took this as the minimum age for classification designated as follows. (1) 'Black': affected, or if unaffected, with both parents affected, that is, of presumptive homozygous recessive *ss* genotype; (2) 'grey': unaffected, but with one parent affected or with affected progeny, that is, of presumed heterozygous recessive *sS* genotype; (3) 'white': unaffected, with neither parent affected and with no affected progeny, that is, of probable homozygous dominant *SS* genotype, although a proportion, the progeny of grey × grey matings, will be heterozygotes; (4) 'proven whites': rams shown by test-mating to be free of the presumptive scrapie allele, that is, of 'proven' double-dominant genotype. The progeny generations sired without interruption by 'proven' rams are designated F_1W, F_2W and so on. Assuming the original F_0 dams to be of the three genotypes in equal numbers, then the chance that an animal of the F_5W–F_6W generations carries the scrapie allele will be about 1 in 100, and of the F_8W–F_9W generations about 1 in 1,000.

Rams were test-mated with 'black' and 'grey' ewes to provide sufficient progeny for at least 10 offspring out of 'black' ewes or 22 out of 'grey' ewes, or a suitable proportion of each dam category, to be observed for at least 4½ yr. Assuming that only 80% of potential cases of scrapie, the *ss*, manifest the disease by 4½ yr, as a more recent study indicates[13], the probability that an allele-carrying ram in such a sire-progeny group will fail to produce an offspring affected within 4½ yr is $<1\%$. A ram with no affected progeny is assumed not to carry the scrapie allele and is a 'proven white'. Of 105 rams tested in eight flocks, 59 had more than one affected progeny and were discarded; 46 had no affected progeny; of these, 18 have proved to be free of the allele at a level of $P < 0.01$ or less, 3 to $P < 0.05$–0.01, 4 are now under test, and for 17, the data are insufficient to provide an assess-

Table 1 Scrapie among the progeny of affected and unaffected rams born in one flock at different periods over 23 yr

	a		*b*	
Birth cohorts	1951–59	1966–73	1958–59	
Mean annual flock attack rate	11.0%	1.4%	14.9%	
Sires	7 affected	8 affected	4 affected	1 allele-free
Total progeny retained/average life span (yr)	242/4.7	103/5.2	85/4.2	21/7.5
Total affected/affected plus unaffected at age 4½ yr or older	109/195 (56)*	2/71 (3)	45/71 (63)	0/17
Out of 'black' (*ss*) dams	53/56 (95)	1/2 (50)	21/22 (96)	0/10‡,‖
Out of 'grey' (*sS*) dams	42/86 (49)	1/25† (4)	18/35 (51)	0/7‡,¶
Out of 'grey'/'grey' dams	14/39 (36)	—	6/12 (50)	—
Out of 'white' (*SS*) dams	0/14§ (0)	0/44 (0)	0/2 (0)	—

* % Affected with scrapie.
† Under-manifestation.
‡ Two in each group left affected progeny in the F_2 generation.
§ Nine brains examined histologically and found free of lesions at age 9½–10 yr.
‖ Nine brains examined histologically and found free of lesions at age 7–10½ yr.
¶ Six brains examined histologically and found free of lesions at age 7–10½ yr.

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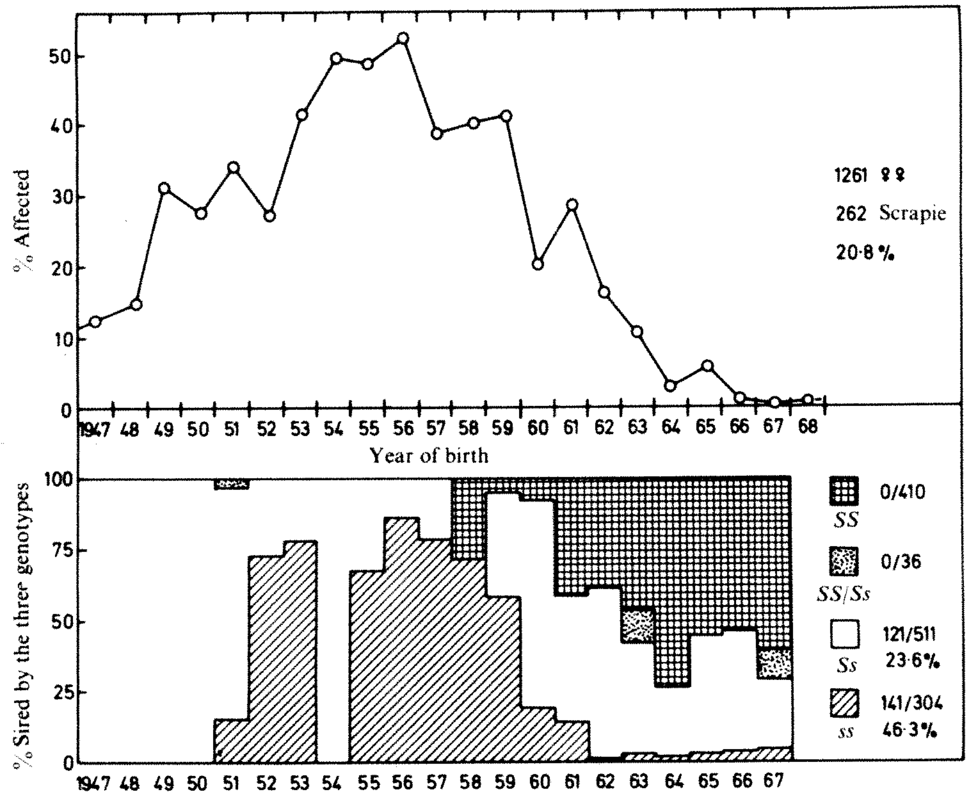

Fig. 1 The scrapie attack rate in the females of one flock for the period 1947–67 shown by annual birth cohorts in the curve (above) and according to the scrapie genotypes of their sires in the histogram (below).

ment, whereas in 4 cases test-mating was abandoned for other unrelated reasons. The 18 'proven white' rams were born and reared in 10 flocks, and were at stud in 3 flocks, which had scrapie present throughout the rams' lives. Ewes from 15 flocks, some with scrapie and some not, have been sent to these rams for mating.

This breeding programme confirmed three predictions of the genetic hypothesis[2,13–15]. (1) That no progeny of a 'proven white' ram should develop scrapie under any natural circumstances. 1,223 Progeny, 1,167 female and 56 male, of the 18 'proven white' rams were born and reared to adults in 15 flocks. 825 Females and 12 males were observed to $4\frac{1}{2}$ yr or older, many to 7 yr, the oldest being $13\frac{1}{2}$ yr. No case of scrapie has occurred in these sheep, although all 15 flocks contained affected animals during some part of the observation period, 1958–78. Sixty-three progeny were out of dams affected during their gestation or suckling, or manifesting scrapie within 2 yr; 46 were observed to $4\frac{1}{2}$ yr or older, with 25 observed to $7\frac{1}{2}$ yr and some to 11 yr; no clinical signs of scrapie were observed. Eight of the oldest animals, which had lived continuously in contact with clinical scrapie, showed no microscopical evidence of scrapie or of the common nonspecific senile changes. Data on the progeny of the first 'proven white' ram, kept in a high incidence environment, are shown in Table 1*b*. (2) That cases will reappear in the F_2 backcrosses of 'grey' parents sired by 'proven white' rams. In the high incidence flock cited in Table 1, matings of two heterozygous F_1W rams with known allele-carriers provided 21 progeny observed to $4\frac{1}{2}$ yr or older, of which five developed scrapie. (3) That consecutive top crosses of 'proven rams' should so reduce the scrapie-allele frequency that the disease will disappear. 308 Females and 18 males of the F_2W–F_6W generations remain under observation and free of clinical scrapie. Figure 1 shows the gradual disappearance of scrapie from one severely affected flock following the retention of the progeny of 'proven white' rams. No case has occurred in the main flock since the 1968 cohort, although two females retained for ram test-mating and six introduced stud rams were affected up to 1976. One F_5W ram carried the allele, derived from an affected great-great-great-grandmother and passed 'silently' without clinical illness through the intervening generations. In the breeder group, which has been using some 'proven white' rams and their sons since 1962, continuous data for 1953–78 are available in 13–18 flocks with 1,900–2,900 flock ewes. The attack rate was 0.4% in 1953–54, reached a peak of 3.2% (85 cases) in 1963–64, and then declined gradually to 0.5% in 1972–73, and 0.2% in 1976–77, with no new cases in home-bred sheep since 1977.

Table 2 Occurrence of scrapie by age $4\frac{1}{2}$ yr among the progeny of the nine reciprocal matings of the three presumptive scrapie genotypes

		No. of progeny		
		Total	Affected with scrapie	
No. of sires	Parents' genotypes ♂♂ × ♀♀	affected plus unaffected to $4\frac{1}{2}$ yr	Observed	Expected†
a				
7	*ss* × *ss*	56	48	48
	ss × *Ss*	124	57	53
11	*Ss* × *ss*	46	23	20
	Ss × *Ss*	106	18	22
7	*SS* × *ss*	39	0	0
	SS × *Ss*	7	0	0
	SS × *SS**	159	0	0
b				
8	*ss* × *SS**	156	0	0
2	*Ss* × *SS**	23	0	0

* Presumed very low *s* allele frequency based on family, flock and breeder group records over 20 yr.

† Based on assumption of 85% of total manifestation completed by age $4\frac{1}{2}$ yr (ref. 2).

Figure 1 also shows that the recessive gene concept provided precise explanations of the variable occurrence of the disorder, given the genotypes of the dams. Table 1 amplifies this analysis during periods of high and low flock attack rates. The agreement with the mendelian predictions is very close. This is confirmed by the summary of the nine reciprocal matings shown in Table 2, those in section *a* being from the flock in Fig. 1, and those in section *b* from three other flocks of the same breeder-group and free of scrapie since 1953.

After a 20-yr breeding programme based on my genetic hypothesis, scrapie has disappeared in the breeder group as predicted. Although our affected sheep carried the scrapie

TSEPA, I doubt that it is ever communicable outside the laboratory. There is no good evidence for dissemination of the disease by a communicable agent[10,11], by placental cannibalism[17], by vertical transmission from the dam[18], or through the action of a dominant susceptibility gene[19,20]. I conclude that in the sheep population studied and probably in most farm sheep in Britain (1) natural scrapie is determined, primarily, by an autosomal recessive gene expressed fully in both sexes; (2) no F_1 progeny of a 'proven' allele-free ram will develop scrapie, whatever the scrapie status of its dam or its exposure to cases of the disease; (3) the scrapie attack rates among the nine progeny groups derived from the reciprocal crosses of the three parental genotypes will conform to the predictions of Mendel's law; (4) the practical control of the disease, and its elimination if desired, may be achieved by the use of allele-free rams and the retention of their progeny; (5) the dissemination of natural scrapie does not depend on a naturally communicable parasitic agent; and (6) the hypothesis most consistent with present evidence[2,10,15,16] is that the scrapie TSEPA is formed *de novo* in each affected animal by the metabolic activity of the natural recessive gene[2,16]. A more detailed account of this work will be published elsewhere[21].

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In vitro transformation of cultured cells from *Nicotiana tabacum* by *Agrobacterium tumefaciens*

CROWN GALL, the plant tumour of many dicotyledonous and some monocotyledonous species, is caused by *Agrobacterium tumefaciens* infection after wounding[1]. Crown gall cells, unlike cells derived from healthy plants, have the ability to proliferate indefinitely in culture without the growth hormones auxin and cytokinin[2]. The properties of the tumours, such as morphology (rough or smooth type) and the synthesis of the unusual amino-acid derivatives octopine or nopaline, are determined by the tumour-inducing (TI) plasmid present in the virulent bacterial strains[3,4]. The presence of at least a part of the plasmid has been demonstrated in the DNA of the tumour cultures[4]. Transcription of this fragment was also demonstrated in the cells of the tumour tissues[5,6]. These findings seem to confirm the involvement of the TI plasmid in crown gall disease. However, it is not known if introduction of the TI plasmid alone into the plant cells is sufficient to obtain transformation of healthy plant cells into tumour cells. A fundamental approach to this question involves the development of an *in vitro* transformation system which can use TI plasmids of *A. tumefaciens* as vectors of genetic material in experiments to modify the genetic information of plants. We report here the transformation of tobacco cells by virulent strains of *A. tumefaciens*, the isolation and characterisation of

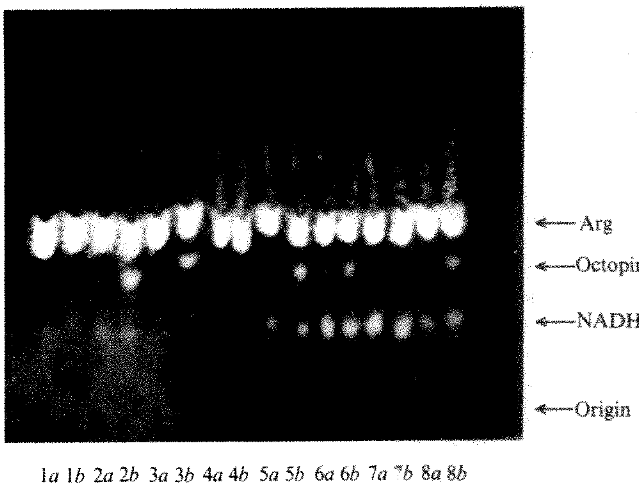

Fig. 1 Electropherogram of the products present in the reaction mixture prepared to detect LpDH activity in extracts of different transformed calli and shoots. The enzyme activity was assayed according to Otten[14]. *a* Channels, reaction mixture at $t = 0$; *b* channels, reaction mixture at $t = 60$ min; 1, *N. tabacum* SR_1 shoot (control); 2, SR_1–4013–1 shoot; 3, SR_1–4013–3 shoot; 4, SR_1–4013–28 shoot; 5, SR_1–4013–1 callus; 6, SR_1–4013–3 callus; 7, SR_1–401328 callus; 8, B_6S_3 crown gall tumour callus (control).

Table 1 Number of colonies in hormone-free medium after incubation of SR_1 cells with different strains of *Agrobacterium*

Bacterial strain	No. of plates seeded	No. of colonies	Frequency (approximate)
LBA 4001 (p)	4	4	1×10^{-4}
LBA 4013 (p)	9	34	6×10^{-4}
LBA 4011 (c)	6	—	—
LBA 305 (p)	6	1	1.5×10^{-5}
LBA 301 (np)	6	—	—

To obtain SR_1 cells, protoplasts were isolated[9] from sterile shoots[10] and cultured in K_3 medium[9,10] supplemented with naphthalene acetic acid (0.1 mg l^{-1}) and kinetin (0.2 mg l^{-1})[12]. The protoplasts were first kept in the dark for 24 h followed by 48 h at 2,000 lx. The cells were mixed with bacteria in a concentration of 10^5 cells per ml and 10^7 bacteria per ml. They were incubated at 20 °C for 32 h in K_3 medium. The light intensity during this incubation and all further treatments was 2,000 lx. After incubation free bacteria were removed by repeated washing and the tobacco cells (10^4 per ml) were cultured in K_3 medium supplemented with vancomycin (250 mg l^{-1}), carbenicillin (200 mg l^{-1}) and streptomycin (200 mg l^{-1}). Three weeks later the colonies were collected by centrifugation, washed with hormone-free medium, and plated in Petri dishes (10 cm) into hormone-free K_3 medium solidified with 0.5% agar. The concentration was approximately 10^4 colonies per plate. This solid medium contained the same antibiotics as the previous liquid medium, plus erythromycin (200 mg l^{-1}). Bacterial strains: LBA 4001, wild type *A. tumefaciens* Ach 5; LBA 4013, mutant of Ach 5 with a TI plasmid that is derepressed for transfer and constitutive for the octopine enzyme; LBA 4011, an avirulent derivative of Ach 5, cured of TI plasmid by growth at 37 °C, rifampicin resistant; LBA 301, wild type *A. radiobacter* S1005, non-pathogenic; LBA 305, S1005, converted to a pathogen by the introduction of a B6 octopine TI plasmid. p, Pathogen, carrying TI-plasmid; c, plasmid free, non-pathogen; np, non-pathogen.

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transformed calli and the regeneration of shoots with a distinct tumour marker.

Preliminary studies with mixed populations of healthy tobacco cells and B_6S_3 crown gall tumour cells were carried out to define optimal conditions for recovery of transformed cells. These experiments were based on the properties of the tumour cells, such as hormone-independent growth and the synthesis of octopine, and on the properties of the *Nicotiana tabacum* SR_1 cells, such as hormone-dependent growth, streptomycin resistance and formation of green colonies in culture medium. It was found that protoplasts, cells or colonies of B_6S_3 cells were able to grow in hormone-free medium whereas those obtained from the SR_1 plants could not grow in these conditions.

SR_1 protoplasts were isolated from leaves of sterile shoots and cultured for 3 d in medium with hormones. During this period the protoplasts regenerated new cell walls and a few underwent the first cell division.

The bacterial strains used were the virulent strains LBA 4013, LBA 4001 and LBA 305, and the non-virulent strains LBA 4011 and LBA 301; the strains are specified in the legend to Table 1. For transformation, the SR_1 cells were mixed with bacteria and incubated for 32 h. During this incubation, aggregation of cells and bacteria was observed. Cells aggregated within a few hours when LBA 4013 bacteria were present, whereas aggregation in the presence of LBA 4001 or LBA 4011 was observed only after at least 5 h. Incubation with LBA 305 and LBA 301 resulted in a slight aggregation that was observed only after 24 h. After incubation, the cells were washed and cultured in liquid medium supplemented with hormones, followed by subculture in solid medium without hormones. This selective medium allowed only hormone-independent cells to grow. Colonies, which were scored after a culture period of 7 weeks (Table 1), were only observed when SR_1 cells were incubated with virulent strains of *A. tumefaciens* and not in control plates when SR_1 cells were incubated with *A. tumefaciens* strains not containing the TI plasmid either after curing or as an original non-pathogenic isolate.

Colonies were isolated and subcultured for further growth and characterisation on selective and non-selective medium; their properties are listed in Table 2. Most of the SR_1–4013 calli retained the ability to proliferate hormone independently after subsequent passages on hormone-free medium. Some of these calli demonstrated lysopine dehydrogenase (LpDH) activity whereas others did not. It is obvious that the group of calli which are hormone independent and LpDH positive is the most conspicuous, because it contains the highest number of calli in which the two tumour markers are observed. Another interesting callus is the SR_1–4013–5, which apparently lost its ability to proliferate hormone independently even though LpDH activity was present.

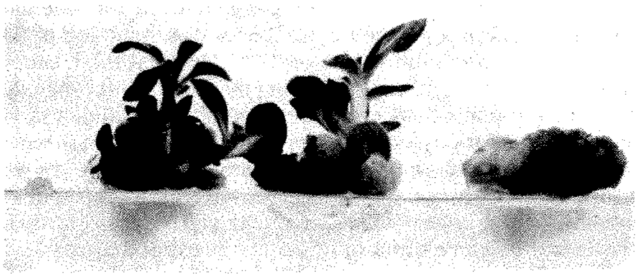

Fig. 2 Five-months-old differentiating SR_1–4013–1 callus obtained after incubation of SR_1 cells with *A. tumefaciens* LBA 4013. The callus was isolated from K_3 medium without hormones and cultured for 6 passages in LS medium without hormones, solidified with 0.8% agar at 2,000 lx.

Before comparing the tumorous calli obtained from these *in vitro* transformation experiments with calli obtained *in vivo* after wounding and infection of plants with the same virulent bacterial strains, it is worth mentioning that the *in vivo* calli, which we have isolated with the LBA 4013, always contain both tumour markers. These calli have been cloned and subcultured for more than 2 yr during which time no reversion to hormone dependence or LpDH deficiency has been observed. This indicates that the loss of tumour markers in callus tissues is a specific phenomenon for those tumours obtained after *in vitro* transformation. Similar observations, concerning the separation of these tumour markers in callus cultures, have not been published before.

The most likely explanation for the observation that during subculture not all the hormone-independent calli contained LpDH activity and for the detection of enzyme activity in hormone-dependent calli is segregation of genes coding for these different properties of the tumour cells. This phenomenon is now being investigated by experiments involving the isolation of TI DNA from the calli and its characterisation using the Southern blot procedure[8]. A correlation between the presence of certain parts of the TI DNA and the expression of certain tumour markers has recently been found in *in vivo* transformation experiments by the isolation of virulent plasmid deletion mutants of *A. tumefaciens* which have lost the property to establish octopine synthesis in tumours which they induce[15,16].

Although most calli were isolated after incubation with the LBA 4013 strain, Tables 1 and 2 show that four calli originated from the use of LBA 4001 and one callus from the LBA 305. From control experiments, in which the non-virulent strains LBA 4011 and LBA 301 were used, no colonies have been isolated (Table 1). The four SR_1–4001 calli did not have LpDH activity; two of them even lost their hormone-independent

Table 2 Characterisation of cell strains obtained after incubation of SR_1 cells with pathogenic strain of *Agrobacterium*

Cell–bacterium combination	Callus no.	Streptomycin resistance	Growth without hormones	LpDH activity	Regeneration
SR_1–4013	1; 3; 22	+	+	+	+
	4; 10; 13; 14; 15 17; 18; 19; 20; 21; 23; 24; 25; 26; 29; 30; 31; 33; 34	+	+	+	−
	11; 28	+	+	−	+
	6; 7; 32	+	+	−	−
	16	−	+	−	−
	5	+	−	+	−
	2; 8; 9; 12; 27	+	−	−	−
SR_1–4001	1; 2	+	+	−	−
	3; 4	+	−	−	−
SR_1–305	1	+	+	−	−

Colonies were isolated and subcultured in hormone-free LS medium[13]. Their streptomycin resistance was tested by culturing them in LS medium with hormones and streptomycin (1 mg ml^{-1}). The media were solidified with 0.5% agar. The light intensity was 2,000 lx.

character. The SR_1–305 callus seemed to be hormone independent and negative for LpDH activity. Calli which were both hormone dependent and LpDH negative were recovered after incubation with the LBA 4013 and the LBA 4001 bacterial strains. Although tumour markers can no longer be detected in these calli after four passages, they were isolated as hormone-independent colonies and obtained from the selective medium from those cultures of cells incubated with virulent bacterial strains.

Our results suggest that the calli in which tumour markers were no longer detectable after four passages have lost their transformed character by loss of the tumour-determining genes during subsequent cell divisions. The significance of these calli is also being studied.

The different numbers of transformed colonies observed by using the three virulent bacterial strains (Table 1) indicate a high efficiency of transformation by the LBA 4013, as compared with the LBA 4001 and the LBA 305 strains. This might be related to the ability of the LBA 4013 to transmit the TI plasmid to other bacteria in a high frequency[15]. Further experiments will show whether these differences are statistically significant.

The colour of the calli varied from white to green (results not shown), but a positive correlation between colour and the other phenotypic traits could not be established. The same is true of the capacity to produce shoots (Table 2). These arose on two LpDH-negative calli, whereas three LpDH-positive calli could also regenerate shoots. These shoots were also LpDH positive (Fig. 1). Figure 2 shows one of the LpDH-positive calli with the shoots. The morphology of the LpDH-positive shoots differs from normal SR_1 shoots in that the transformed shoots show an extensive lateral shoot formation and almost no size growth. The stems and the leaves are relatively thick, and there is almost no apical dominance. Roots cannot be induced. Regeneration in the way described for the *in vitro* transformed SR_1 calli has not been observed on tumorous calli obtained after *in vivo* transformation of shoots of *N. tabacum*.

Extension of the observations presented here, using purified TI-plasmid DNA as the transforming vehicle, is now being carried out. Preliminary results from these experiments indicate that TI DNA alone is sufficient for tumour induction in cultured plant cells. The successful transformation of individual plant cells by *A. tumefaciens*, its regeneration into shoots in which tumour markers are expressed, and the possibility of isolating new bacterial strains containing recombinant TI plasmids[17] provide new tools for the introduction of foreign genetic information into plant species.

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Rat × rat hybrid myelomas and a monoclonal anti-Fd portion of mouse IgG

MONOCLONAL antibodies can be produced by cultures of permanent cell lines derived by fusion of suitable myelomas with spleen cells from immunised animals[1]. So far, mainly two mouse myelomas, X63-Ag8 and NSI/1-Ag4.1, have been used for this purpose[1,2]. When cells from immunised mice are used in the fusion, the mouse × mouse hybrid myelomas can be grown as tumours in appropriate mouse strains. We describe here the preparation and use of a new clone of a rat myeloma, which is suitable for the derivation of rat × rat hybrid myelomas producing specific rat antibodies. The spent medium of hybrid myeloma cultures usually contains 1–20 $\mu g\ ml^{-1}$ antibody. The serum and ascites of the tumour-bearing mice, with few exceptions, yield 1–20 $mg\ ml^{-1}$ of antibody, which is 1,000 times more concentrated and very convenient for larger preparations.

There are several reasons for preferring rats to mice for the production of monoclonal antibodies. The yield of serum and ascitic fluid is about 10 times better in tumour-bearing rats than mice, an overwhelming economic advantage in large scale production. Although there is a marginal increase in the generation time (10 weeks in the rat and 8 in the mouse) the rat litters are usually large. The derivation of hybrid myelomas using cells from immunised rats is a very successful procedure for deriving rat alloantibodies or anti-mouse xenoantibodies[3,4], but because the derived lines are of mouse × rat origin the growth of tumours *in vivo* in either rats or mice requires rather elaborate procedures or special strains. Clearly, rat × rat fusion would be advantageous for making xenogeneic anti-mouse and allogeneic anti-rat antibodies, as well as others, where large scale production is anticipated. This requires a suitable parental rat myeloma line.

Myeloma tumours in the Lou strain of rats have been available for some time[5]. One of them, R210, has been grown in culture and an azaguanine resistant mutant (210.RCY3.Ag1) prepared. It secretes κ chains and was used in the first successful fusion between two myelomas[6]. When tested for its ability to fuse to normal or immunised rat spleens it gave disappointing results. It was twice subcloned in soft agar and the clone finally selected for further study was called Y3-Ag1.2.3. It gave no revertants when batches of 10^7 cells were tested. Fusion efficiencies were at first disappointing and the line was grown continuously in a spinner flask and tested for fusion efficiency at

Table 1 Species specificity of YA2/40

Species	Serum dilution giving 50% inhibition
Mouse	1:10,000
Guinea pig	1:2,000
Rabbit	1.40
Horse	>1:10*
Human	>1:10*
Rat	>1:0*

Inhibition of [^{3}H]YA2/40 binding to Sp2 coated SRBC was as described in Fig. 2.

* No inhibition detected at 1:10.

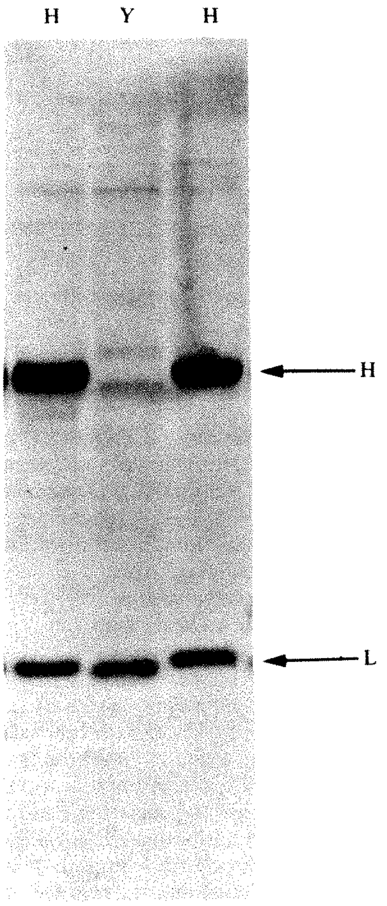


Fig. 1 Analysis of Ig secreted by the rat hybrid myeloma clone YA2/40 H(LK). Cells were incubated for 24 h with ^{14}C-lysine and supernatants were analysed[2] by SDS-polyacrylamide (10%) gel electrophoresis after total reduction. Y refers to Y3-Ag1.2.3 (control); H to Y2/40 H(LK) clone. DA rats were immunised by five footpad injections at 3-week intervals with 100 μg of mouse IgG in complete Freund's adjuvant and boosted by intravenous injection without adjuvant 4 days before fusion. Supernatants were tested 13–15 days after fusion by indirect haemagglutination as follows: 2.5×10^8 SRBC were coated by incubation for 1 h at room temperature with 5 ml of culture supernatants of anti-SRBC hybrid myelomas[2] minimally diluted to avoid agglutination. They were centrifuged and the pellet resuspended in 2.5 ml of phosphate-buffered saline (PBS). Aliquots of 25 μl were distributed in V-bottom microtitre trays and 25 μl of supernatant added to each well. After 1 h incubation the plate was centrifuged at 200*g* for 4 min and then kept in a 45 ° slope for 30–60 min. Culture YA2/40 was selected in this way and cloned twice in soft agar.

different times. The first successful fusion was obtained after 5 months of continuous growth. Using about 5×10^7 Y3 cells and 10^8 spleen cells the fusion efficiency has improved to a stage where it is comparable to the mouse lines. The Y3 rat cells and their hybrid derivatives are much smaller in appearance than the mouse X63-Ag8 cells. Early stages of hybrid growth are less obvious under the microscope. Y3 cells grow very well on DMM supplemented with 5% or less fetal calf serum and the clonability in soft agar is very good. The line and the hybrids derived from it give rise to rather diffuse clones, instead of the tight ones given by mouse × mouse hybrids.

Our first successful derivation of a rat monoclonal antibody by fusion of Y3-Ag1.2.3 was with spleen cells from a DA strain rat which had been hyperimmunised with mouse IgG (see Fig. 1 legend). The fusion protocol used polyethylene glycol 1500 as previously described[3]. The hybrid culture was divided into 48 subcultures. In this experiment only 6 showed hybrid growth. The spent medium of the hybrid cultures was tested by indirect haemagglutination of sheep red blood cells (SRBC) which had been sensitised with different mouse monoclonal antibodies specific for SRBC[2]. One hybrid was clearly positive when the SRBC were coated with Sp3 (an IgG1) but negative when coated with Sp2 (an IgG2b) or Sp1 (IgM). The positive hybrid was subsequently cloned in soft agar and 8 individual clones were selected. All were found to be positive by the same test. The antibody secreted by each clone was internally labelled with ^{14}C-lysine and analysed by SDS-polyacrylamide gel electrophoresis and isoelectric focusing (Fig. 1) (data not shown). All appeared to be identical, revealing a heavy chain band of about 50,000 MW and a single band present in the light chain zone. We were not able to demonstrate the presence of the two expected light chains (the myeloma parental and the antibody specific light chains). It is quite possible that both have very similar mobilities. Three of the above clones were cloned again to ensure purity and increase stability. One of these subclones was named YA2/40 H(LK) and used in all the experiments described below.

Binding of internally labelled [^{3}H-Lys]YA2/40 to cells coated with different classes of anti-SRBC antibody showed that YA2/40 recognised mouse IgG but not IgM. Binding to Sp1 (IgM) coated cells gave the same value as the background binding to uncoated cells. Binding to Sp3 (IgG1) and Sp2 (IgG2) coated cells was 4.9 and 35 times background respectively, a difference which correlates with the number of antigenic sites recognised by Sp2 and Sp3 (the number of antigenic sites recognised by Sp1 is intermediate between Sp2 and Sp3; C. D. Wilde, A. Williams and C. M., unpublished). The failure of YA2/40 to agglutinate Sp2 coated red cells although it agglutinated Sp3 coated cells (see above) remains unexplained.

Coating with Sp2 gives a sensitive procedure to assay for binding of [^{3}H]YA2/40, and further specificity studies were done by inhibition of this binding. These studies confirmed that YA2/40 recognised individual proteins of both $\gamma 1$ and $\gamma 2$ subclasses of IgG (Fig. 2*a*). Inhibition by an IgM was at least 50-fold less effective. At high concentrations of protein some inhibition was observed, probably because of IgG impurities in the preparation. The antigenic determinant in the IgG1 is located in the Fd region for the following reasons. The inhibition of binding by MOPC 21 is equally effective with its variant IF1 (a mutant lacking the CH3 domain[7]) and with the $F(ab')_2$ fragment of MOPC 21 which lacks the CH2 and CH3 domains[8]. In both cases the inhibitory titre on a molar basis was similar to the intact protein and slightly higher on a per weight basis (Fig. 2*b*). On the other hand the IF2 mutant protein (which lacks the CH1 domain) was a very poor inhibitor. The residual inhibition is probably due to a trace of IgG present in the IF2 purified from serum of tumour-bearing mice which can be detected by SDS-polyacrylamide gel electrophoresis. No intact H-chain was present in the $F(ab')_2$ while a trace impurity of 50,000 MW component was detected in the IF2 preparation (data not shown).

As regards species specificity YA2/40 seems to be completely negative towards human, horse or rat, but shows some degree of cross-reaction to rabbit serum. On the other hand it is inhibited quite effectively by guinea pig serum. This pattern of cross-

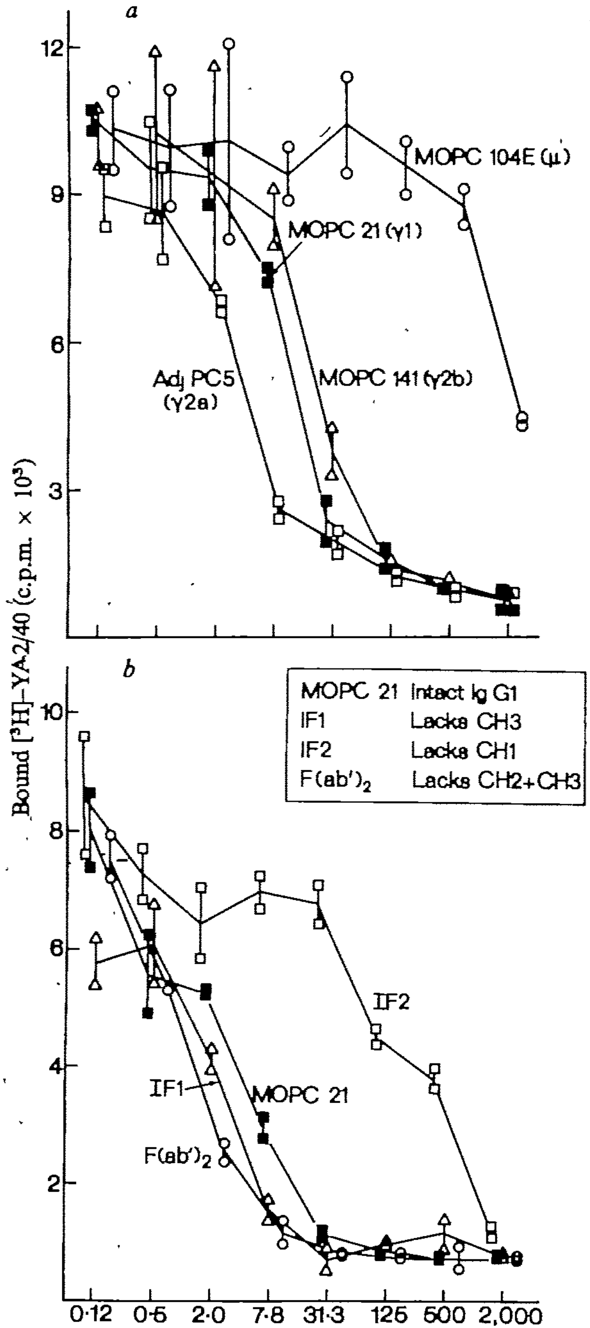
Table 2 Preferential fixation of the hybrid immunoglobulin secretion phenotype following fusion with the rat myeloma line Y3-Ag1.2.3

No. of cultures*	96
Cultures showing growth of hybrids	17
Secretion of Y3-like light chain (only 12 tested)†	11
Secretion of Ig chains different from Y3†	11
Secretion of Y3 light chain in the absence of Ig chains	1

* Y3-Ag1.2.3 cells were fused to spleen cells from an immunised donor (DA rat immunised with cells from an AO rat). The culture was divided into 96 wells and allowed to grow for 4 weeks in selective medium.

† Tested by SDS-polyacrylamide gel electrophoresis of secreted products. Five of the cultures had insufficient numbers of cells for a meaningful test.

Fig. 2 Inhibition of YA2/40 binding to IgG coated cells by other immunoglobulins. 1.2×10^6 SRBC coated with Sp2/HL (IgG2b anti-SRBC) in 10 µl of PBS were distributed in U-shaped microtitre plates. After addition of 20 µl of inhibitor protein solution and 10 µl of ^{3}H-lysine-labelled YA2/40 (170,000 in *a*, 85,000 c.p.m. in *b*) and incubation at 4° C for 1 h, the cells were washed three times with cold PBS, the pellet lysed with 50 µl of H_2O and transferred to a scintillation vial. Counting was in 2 ml of scintillant (5 g PPO, 0.2 g POPOP, 540 g Triton X-100 and toluene to 1 l final volume). All proteins[8] were purified from serum by $(NH_4)_2SO_4$ precipitation and DEAE chromatography. $F(ab')_2$ fragment was prepared as before[7] and purified by Sephadex G-100 ultrafiltration. Internally labelled YA2/40 protein was prepared as follows: 5×10^6 YA2/40 H(LK) cells were incubated in 5 ml of lysine-free medium containing 0.2 mCi ml^{-1} ^{3}H-lysine; after 8 h incubation at 37 °C, 5×10^6 more cells were added and the incubation continued for another 12 h. The supernatant was collected and dialysed three times against 0.5 l of PBS.



reactivity indicates that the antigenic determinant involves a similarity of local structures in guinea pig and the different mouse subclasses which, however, differ completely from the human, horse and rat. A close inspection of the sequences of the CH1 of mouse γ1 (ref. 9), mouse γ2*a* (ref. 10), guinea pig γ2 (ref. 11), rabbit[12] and man γ1 (ref. 13) reveals few examples of such a pattern of sequence similarity. The most interesting sequence involves residues 209 (Ser in mouse and guinea pig, Asn in human), 206 (Pro or Ala in mouse and guinea pig, Lys in human) and 204 (Ala in mouse and guinea pig, Asn in human). This is a cluster of residues in an exposed corner near the V–C domains boundary in the tertiary structure of the Fab[14]. It is important to keep in mind that structures in Fab other than CH1 could be involved in the antigenic recognition, the deletion of CH1 having a general effect on the tertiary structure of the Fab. Elucidation of the rat and horse sequences could throw further light on the precise nature of YA2/40 determinant. YA2/40 H(LK) cells grow as solid tumours in F1 (Lou × DA) rats and the serum of the tumour-bearing rats contains 10–15 mg ml^{-1} IgG as measured by radial immunodiffusion, giving rise to a prominant myeloma band component in cellulose acetate electrophoresis. The tumour could also be grown as an ascitic tumour. This was achieved by an intraperitoneal injection of 0.5 ml of pristane about 2 weeks before the intraperitoneal injection of 5×10^7 cells (P. Wright, unpublished).

In a subsequent experiment, hybrids obtained from this line fused with spleen cells of an immunised rat were analysed by their ability to secrete Ig different from the myeloma parent. This was done by SDS-polyacrylamide gel electrophoretic analysis of the secreted products. Out of 12 cultures tested (all probably monoclonal) 11 secreted Ig chains and only 1 failed to secrete chains different from the myeloma parent. So, well over 60% of the tested cultures (and even of total number of growing cultures) secreted new immunoglobulin. This value is even higher than that obtained for the mouse system[2] and is in line with the hypothesis that the fusion between a myeloma cell and the heterogeneous population of cells present in the spleen is a highly selective event which favours the rescue of active antibody secreting phenotype. In a wider context it indicates that for the recovery of a transient differentiated function in the form of an immortal cell line, it is best to use a fusing parental cell which is phenotypically close to the normal phenotype one attempts to immortalise.

In conclusion, 210RCY3-Ag1.2.3 is a myeloma cell line suitable for the derivation of rat × rat hybrid myelomas. This seems a better alternative than the mouse × mouse hybrids for large scale production of monoclonal antibodies of potential general use in research, medicine and industry. The anti-mouse IgG monoclonal antibody described here is an example of such hybrids.

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Continuous cell lines with specific killer T-cell activity

THE establishment of continuous cell lines with effector T-cell activity is an obvious starting point in the isolation and characterisation of the T-cell receptor complex. Although nature provided the plasmacytomas which played a key part in elucidating B-cell immunoglobulin structure, there are no known examples of naturally occurring lymphomas with effector T-cell activity. Initial success with fusions between plasma cells and plasmacytomas encouraged extrapolation of this technique to T cells and thymic lymphomas[1], but few promising results have so far been obtained. We have approached this problem by using the carcinogenic properties of mutagens to transform T lymphocytes responding in mixed lymphocyte culture (MLC), with the aim of isolating cell lines with killer T-cell activity. Here we report our success in the first step of isolating continuous, though antigen-dependent, killer T-cell lines.

We started with C.B20 (H–2^d BALB/c, Ig–1^b congeneic) mouse spleen cells responding to irradiated (3,000 R) C57BL/6 (H–2^b) spleen cells, in culture conditions described by McDonald[2]; 7 d after the second *in vitro* boost, cells were mutagenised (1 h) with (2 μg ml^{-1}) nitrosoguanidine. Mutagenised cells were restimulated twice more, then seeded at 10, 10^2 or 10^3 cells per well in Falcon Micro Test II tissue culture plates and cultured with 10^6 irradiated C57BL/6 spleen cells per well which also served as a feeder layer. Individual wells were scored for growth, and killing capacity was tested by adding 5×10^3 of HAT (hypoxanthine, aminopterin, thymidine)-sensitive ^{51}Cr-labelled EL4.BU target cells (addition of HAT medium after assay killed all remaining EL4 cells leaving HAT-resistant MLC cells to grow). Although some wells had high killing activity, these wells did not show good growth. There were 3 of 384 wells positive for growth from the 10-cell per well plates and 1 of 384 positive from the 10^2-cell per well plates. The four isolates were expanded by weekly stimulation with irradiated C57BL/6 spleen cells and tested for growth requirements as well as killer activity. Each isolate contained only Thy 1.2-positive cells as tested by anti-Thy 1.2 and complement lysis (data not shown).

As shown in Fig. 1, the four isolates have different killer-cell activities. The isolates 100.1 and 10.3 show typical H–$2D^b$ and H–$2K^b$-specific killing, respectively. Isolate 10.2 killed only in the presence of concanavalin A (Con A) or after Con A stimulation, and isolate 10.1 killed only EL4 and not C57BL/6 lipopolysaccharide blast targets. The specificity of killing exactly paralleled that of growth stimulation; for example, 10.3, which killed B10.A (5 R) (K^bD^d) but not B10.HTG (K^dD^b) was stimulated to grow with B10.A (5 R) but not B10.HTG. Isolates 10.2 and 10.1 were tested for growth with C57BL/6, B10.HTG and B10.A (5 R) irradiated spleen. The results show that 10.1 was specifically stimulated by B10.A (5 R) H–$2K^b$ (Fig. 2), although killing activity waned over the 150-d test period (Fig. 1). Isolate 10.2 showed a differential growth response, as C57BL/6 stimulated better than B10.A (5 R), and B10.HTG gave virtually no growth. The complex growth and killing properties of 10.2 have been studied in some detail without revealing any simple explanation; however, this isolate may well provide new insights on Con A-dependent killing. We found that 10.2, stimulated with Con A for 2 d then washed in medium containing α-methyl-D-mannoside, killed EL4 but not P815 (H–2^d) targets. The isolate 10.2 could not be maintained in culture with either Con A or Con A-supernatant as stimulants, as indeed was true of all other isolates. The cytolytic efficiency of these different isolates varied from near normal MLC values (for example, 100.1) to zero (such as 150-d 10.1), suggesting that either the populations have different intrinsic killing efficiencies, or both active and inactive killers exist in the same population. When the isolates

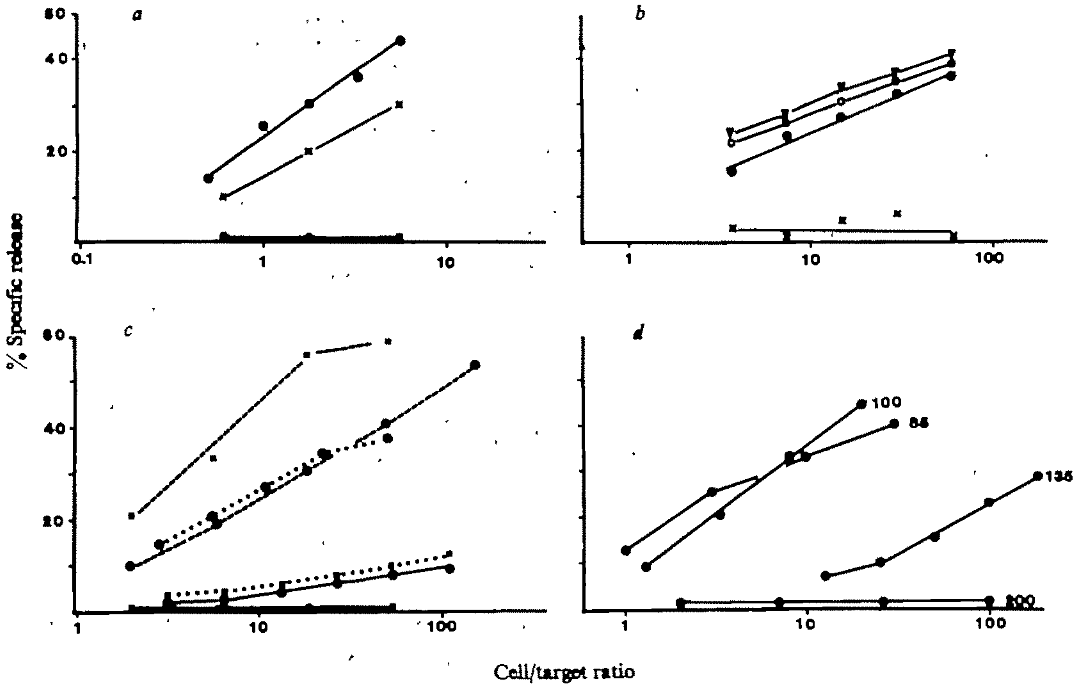

Fig. 1 Cytotoxic activities of four cloned isolates of T cells obtained from a C.B20 (H–2^d) anti-C57BL/6 (H–2^b) one-way MLC after multiple stimulation and mutagenesis *a*, Isolate 100.1; *b*, isolate 10.3; *c*, isolate 10.2, *d*, isolate 10.1. Target cells labelled with ^{51}Cr were EL4 (●), P815 (□) and 2 μg ml^{-1} Con-A blasted spleen cells from the congeneic mouse strains C57BL/6 (H–$2K^b$–D^b) (▽), B10.A (5 R) (H–$2K^b$–D^d) (○) and B10.HTG (H–$2K^d$–D^b) (×). Cytotoxicity assays were carried out in flat-bottomed microtitre plates (Falcon 3040) in a total volume of 0 3 ml with 10^4 ^{51}Cr-labelled target cells and varying numbers of clone effector cells. The amount of ^{51}Cr released during 5 h incubation at 37 °C was calculated on the basis of 0 1-ml samples and the per cent specific target cell lysis was calculated according to the formula. [(experimental c.p.m. – spontaneous c.p.m.)/(total c.p.m. – spontaneous c.p.m.)]100 In experiments using tumour cell and Con A blast targets, spontaneous release was less than 10% and 25–35%, respectively. Isolate 10.2 (*c*) was assayed in the presence (– – – –) or absence (——) of 10 μg ml^{-1} Con A in the cytotoxic test. In one experiment 10.2 cells were cultured in the presence of 2 μg ml^{-1} Con A for 48 h, then washed and incubated in 0.2 M α-methyl-D-mannoside for a further 2 h before testing in the cytotoxicity assay (·····). Isolate 10.1 (*d*) was assayed periodically over 154-d, the numbers indicating the time in days.

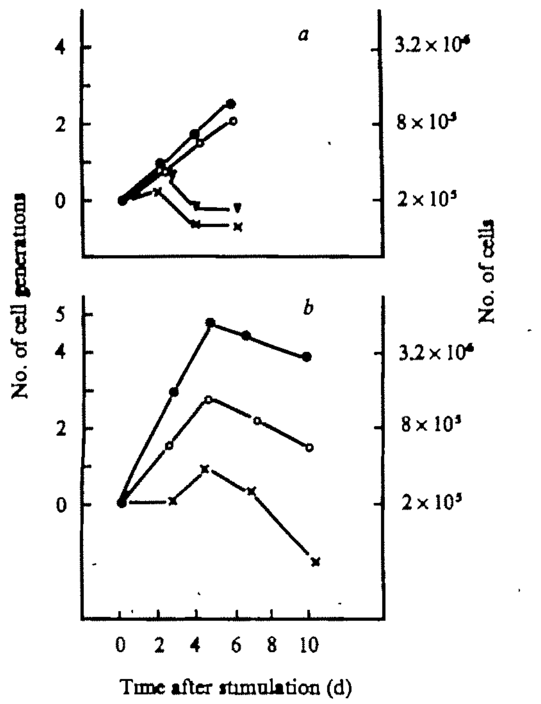

Fig. 2 Isolates 10.1 (*a*) and 10.2 (*b*) were stimulated with 3,000 R irradiated spleen cells from C57BL/6 (●), B10.A (5 R) (○), B10.HTG (×) and C.B20 (▽). In all experiments cells were grown in Dulbecco modified Eagle's medium supplemented with 10% fetal calf serum, 5×10^{-5} 2-mercaptoethanol, 8×10^{-2} M glutamine and non-essential amino acids. In the primary MLC, 2.5×10^{-7} C.B20 spleen cells were mixed with 2.5×10^{7} irradiated C57BL/6 spleen cells in 20 ml medium in Falcon flasks (3012) and incubated for 11 d in flasks standing upright. Subsequent stimulations, whether in flasks, Micro wells or Linbro wells were done at a concentration of about 4×10^{4} stimulator cells mm^{-2} tissue culture surface and 0.01 of the number of responder cells (except in the cloning procedure where the number of responding cells varied). All cultures were incubated at 37°C with an atmosphere of 15% CO_2 in air.

are recloned it will be possible to distinguish these possibilities although our guess would be that low killing efficiency is more conducive to long-term survival of these cells in culture as exemplified by isolates 10.2 and 10.1.

Previous reports from other laboratories have shown that MLC cells can be maintained for long periods[3,4], and in one case killer cells were 'cloned' in agar[5]. The use of soft agar to clone MLC cells was possible because cells could be maintained by Con A-supernatant stimulants, whereas in our case MLC cells require cellular stimulants and cannot be seeded in soft agar. The isolates we have obtained after growing MLC cells at limiting dilution are certainly clonal with respect to both the minimal growth unit and specificity; however, it is still impossible to conclude rigorously that a growth unit is in fact a single T-killer cell. Work is in progress to further mutagenise the isolates for the selection of antigen-independent killer T cells which can be formally cloned and studied as a homogeneous population of effector T cells. Recently, we have been able to use a more efficient carcinogenesis technique (developed by P. Patek and J. Collins, personal communication), which is based on continuous exposure of cultures to methylcholanthrene.

The ability to clone MLC responses seems to depend on prior mutagenesis, and with the techniques described here it is relatively straightforward to isolate growing clones of effector T killers. Further experiments are in progress to test if these killer-cell clones can act as abnormal suppressor cells in the system recently described by Epstein and Cohn[6].

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Effects of *S*-lactoylglutathione and inhibitors of glyoxalase I on histamine release from human leukocytes

THE enzyme responsible for the conversion of methylglyoxal to lactic acid by animal tissues was first named glyoxalase[1,2]. It was later shown that there are two glyoxalase enzymes[3]. The first (glyoxalase I) converts methylglyoxal and reduced glutathione to *S*-lactoylglutathione and the second (glyoxalase II) converts this compound to D-lactic acid, regenerating glutathione in the process. These enzymes are very widely distributed[4] and as yet have no clearly defined function(s). I describe here the enhancement of anti-IgE-induced histamine release by *S*-lactoylglutathione and its inhibition by an inhibitor of and an alternative substrate for glyoxalase I. These two compounds should, of course, decrease the production of endogenous *S*-lactoylglutathione. These experiments were undertaken for two reasons. First, a study from my laboratory has provided evidence that *S*-lactoylglutathione modulates microtubule assembly *in vitro*[5] while the release of histamine from leukocytes is a secretory process thought to require an intact microtubule system[6,7]. Second, concanavalin A has been shown to cause histamine release from human leukocytes[8,9] and has also been found to activate the two glyoxalase enzymes in lymphocytes and polymorphonuclear leukocytes[10].

Leukocytes prepared from the blood of normal donors by dextran sedimentation as previously described[11] were subjected to hypotonic lysis (18 ml of ice-cold distilled water for 20 s followed by 2 ml of 9% NaCl) to remove contaminating red blood cells. They were incubated with anti-IgE and other compounds for 45 min at 37 °C in a balanced salt solution consisting of (mM): NaCl, 110; KCl, 5.0; $CaCl_2$, 0.6; $MgCl_2$, 1.0; and PIPES buffer, 25 mM, adjusted to *p*H 7.3 with 40 mM sodium hydroxide. At the end of the incubation period the cells were centrifuged at 1,000*g* for 90 s and histamine released into the medium was determined using an automated extraction procedure[12].

The effects of *S*-lactoylglutathione on histamine release induced by two different levels of anti-IgE from the cells of normal non-atopic donors are shown in Fig. 1. The dose–response curve to anti-IgE is bell-shaped[13] with maximum release varying from zero to approximately 60 or 70% depending on the individual from whom the cells are taken. The lower of the two anti-IgE concentrations studied is below that required for optimum release while the higher concentration is slightly above it. As can be seen, *S*-lactoylglutathione caused a small increase in release from the cells of 6 out of 7 donors at the lower anti-IgE concentration and from the cells of 9 out of 10 donors at the higher anti-IgE level. This enhancement is statistically significant with $P < 0.003$ (Student's paired *t*-test) or $P < 0.001$ (Wilcoxson matched-pairs signed-ranks test).

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The effect of various concentrations of S-lactoylglutathione (0.2–2×10^{-4} M) on histamine release were studied to establish the concentration necessary for optimum enhancement. The dose–response relationship varied with the cells of different individuals. With the cells of three out of five donors, maximum enhancement was seen at an S-lactoylglutathione concentration of 2×10^{-4} M. With the cells of one donor enhancement in the presence of 5×10^{-5} and 2×10^{-4} M S-lactoylglutathione was identical (51 to 59%) and with the fifth donor 5×10^{-5} M S-lactoylglutathione increased release from 60 to 73% while release in the presence of 2×10^{-4} M was only 64%. In the absence of anti-IgE, S-lactoylglutathione did not itself release histamine. As the preparation contained glutathione (Sigma) and this is one of the metabolic products of S-lactoylglutathione, the effect of glutathione (2.5×10^{-4} M) was studied in seven experiments. It never enhanced histamine release and with the cells of two individuals somewhat (5–15%) inhibited release.

To investigate the role of S-lactoylglutathione in histamine release further, two structurally dissimilar compounds both capable of interacting with glyoxalase I and thereby decreasing S-lactoylglutathione formation were studied. The first of these, glutaryl-S-(p-bromobenzyl)-L-cysteinylglycine (PAL6-25-1) is an analogue of glutathione that is not metabolised by the enzymes capable of degrading glutathione[14]. It inhibits glyoxalase I from yeast with a K_I value of 0.32 mM, but apparently binds to a different site from that of glutathione[15]. The second compound, phenylglyoxal (Aldridge), is an analogue of methylglyoxal (Sigma) and is a substrate for glyoxalase I (ref. 16), forming S-mandelylglutathione rather than S-lactoylglutathione. S-Mandelylglutathione is a poor substrate for glyoxalase II, with a V_{max} for this enzyme only a twentieth that of S-lactoylglutathione[17]. As can be seen from Table 1, both PAL6-25-1 and phenylglyoxal inhibited histamine release in a dose-dependent manner. Furthermore, the simultaneous addition of S-lactoylglutathione more than reversed the inhibitory effect of PAL6-25-1 and also partially (donor 1) or completely (donor 2) reversed the inhibition caused by phenylglyoxal. This reversal provides good evidence that PAL6-25-1 inhibits histamine release because it decreases endogenous S-lactoylglutathione formation. Phenylglyoxal is possibly acting in the same way although it is also conceivable that the S-mandelylglutathione formed from phenylglyoxal is an inhibitor of the action of S-lactoylglutathione.

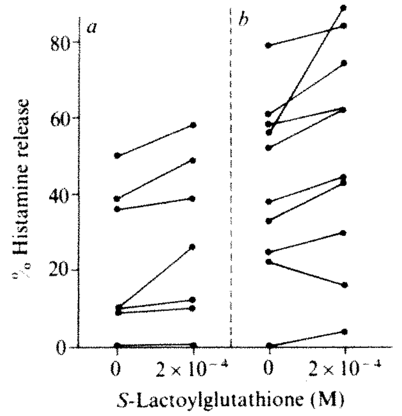

Fig. 1 Effect of S-lactoylglutathione on histamine release from human leukocytes induced by anti-IgE. Leukocytes from normal individuals were incubated in duplicate with anti-IgE (a, 8.3×10^{-3} μg ml^{-1}; b, 0.167 μg ml^{-1}) and with or without S-lactoylglutathione as described in the text. The release reaction was initiated by adding cells prewarmed to 37 °C to tubes already containing all desired compounds. The average difference between duplicate measurements (higher value minus lower value divided by lower value × 100) was 3.8%. S-lactoylglutathione was prepared enzymatically by incubating a neutralised mixture of 20 mM methylglyoxal and 25 mM reduced glutathione with glyoxalase I (4 units ml^{-1}) at room temperature for 1 h. In the presence of excess glutathione the conversion of methylglyoxal to S-lactoylglutatione was complete. The methylglyoxal was purified by steam distillation[16] followed by passage (at a concentration of 50 mM) over Dowex-1.

Table 1 Effects of PAL6-25-1, phenylglyoxal and S-lactoylglutathione on histamine release from human leukocytes

Additions Inhibitor	S-Lactoylglutathione 2×10^{-4} M	% Histamine release (mean ± s.d.) Donor 1	Donor 2
None	−	58 ± 4	64 ± 2
	+	71 ± 3	60 ± 1
PAL6-25-1	−	45 ± 0	51 ± 3
3×10^{-4} M	+	76 ± 2	66 ± 2
PAL6-25-1	−	36 ± 4	41 ± 0
10^{-3} M	+	85 ± 3	70 ± 2
Phenylglyoxal	−	32 ± 1	48 ± 2
10^{-5} M	+	58 ± 4	62 ± 2
Phenylglyoxal	−	9 ± 2	22 ± 1
3×10^{-5} M	+	32 ± 2	60 ± 2
Phenylglyoxal	−	7 ± 1	9 ± 2
10^{-4} M	+	16 ± 3	51 ± 4

Leukocytes from two normal individuals were incubated in triplicate as described in the text. The cells were preincubated for 5 min with PAL6-25-1, phenylglyoxal and/or S-lactoylglutathione after which anti-IgE (final concentration 3.3×10^{-2} μg ml^{-1}) was added and the incubation continued for 45 min. Donor 2 was specifically selected because previous experiments had shown that S-lactoylglutathione had little effect on anti-IgE-induced histamine release from her cells. S-Lactoylglutathione was prepared as described in Fig. 1.

The present findings implicate S-lactoylglutathione as a modulator of anti-IgE-induced histamine release and, as such, represent the first demonstration of an effect of this compound in any secretory system. In addition they are compatible with the general hypothesis that the function of the two glyoxalase enzymes is the regulation of S-lactoylglutathione levels. It is not yet known whether anti-IgE, the stimulus for histamine release, alters the activities of the glyoxalase enzymes and thereby increases S-lactoylglutathione levels in basophils. This is a definite possibility, however, as concanavalin A increases the activities of both glyoxalase enzymes in lymphocytes and polymorphonuclear leukocytes[10] and also causes histamine release from basophils through interaction with IgE[9]. The mechanism whereby S-lactoylglutathione potentiates histamine release is not clear. Because evidence has implicated S-lactoylglutathione in the process of microtubule assembly *in vitro*[5] while histamine release is a microtubule-dependent process[6,7], it is possible that it affects histamine release, in part, by promoting microtubule assembly. It seems unlikely, however, that this is its only mode of action as disruption of microtubules with colchicine does not inhibit release to as large an extent as phenylglyoxal[6,7]. Elucidation of the precise role(s) of S-lactoylglutathione in histamine release will require further study.

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The influence of thymus on the development of MHC restrictions exhibited by T-helper cells

IMMUNE functions depend largely on the activities of peripheral lymphocytes which have been 'processed' in the thymus (T cells). The best characterised activities of these cells, such as *in vitro* cytotoxicity and suppressor and helper capacities in adoptive immunisations seem to be mediated by distinct subpopulations. However, it has emerged that T cells of all subpopulations exhibit a dual recognition faculty, on the one hand, towards entities coded by the major histocompatibility gene complex (MHC) and on the other towards foreign antigens. Without appropriate MHC recognition 'foreignness' may not be noticed, giving rise in various functional assays to what has been termed MHC restriction. For example, in the mouse, T cells may lack cytotoxicity unless they have H–2 region gene products[1], in common with their target cells. T cells which 'help' in antibody responses can only do so fully if the B cells with which they are cooperating have similar I-region coding[2]. These various restrictions were thought to represent the need for interaction of like substances on cell surfaces perhaps to establish propinquity before any subsequent interactions could be initiated. Recently, however, it has been demonstrated[3,4] that irradiated bone marrow chimaeras of the type $A+B \rightarrow (A \times B)F_1$, possessed populations of T cells of A and B origin each of which was capable of interacting with B-cell populations of both A and B origin (A and B are used here purely as symbols for MHC-disparate mouse strains). Thus it seemed whatever the genetic determinacy of the cell, it could, when appropriately conditioned, establish reactions based on identity with either genetically similar or genetically different cells[2,5]. The exact nature of this conditioning began to emerge from experiments of Bevan[6] and Zinkernagel[7] who found that T cells from $(A \times B)F_1 \rightarrow A$ chimaeras could only kill target cells bearing A type MHC determinants, whilst no cytotoxicity towards B targets was possible. Comparable studies gave analogous results for helper T cells[8,9] and recently, it was shown that the events which seem to determine the pattern of H–2 restricted specifities of the T cells concerned with cytotoxicity occur within the thymus gland[7]. We now show that intrathymic conditioning also determines the restriction specificities of T cells involved in helping antibody production.

The experimental design (Table 1 legend) aimed to generate populations of F$_1$ T cells which had differentiated to their T-cell status within parental thymus grafts. In other studies it has been shown that both thymus lobes irradiated[10] as in the present series of experiments, and epithelial thymus grafts[11], are incapable of generating T cells of the graft genotype but are capable of acting as stageing houses for immigrant, unirradiated bone-marrow stem cells. From these and other studies it is a reasonable assumption that the majority of T cells in a thymectomised, irradiated, bone-marrow injected, thymus-grafted mouse such as those in the present studies, will have been processed by the (irradiated) thymus graft.

Thymic chimaeras were constructed using (C3H/He × BALB/c)F$_1$ recipients of irradiated thymus grafts. Three groups of experimental chimaeras were compared. One group received C3H/He (H–2^k), another BALB/c (H–2^d) and the third (C3H/He × BALB/c)F$_1$ thymus grafts. Helper function of spleen T cells from individual donors was then assessed on CBA (H–2^k) and BALB/c (H–2^d) B cells. (C3H/He × BALB/c)F$_1$ T cells which had matured through the influence of a C3H/He thymus tended to cooperate better with CBA B cells; those which had matured through a BALB/c thymus usually showed preference for cooperation with BALB/c B cells (Table 1). Those T cells which had differentiated through a (C3H/He × BALB/c)F$_1$ thymus were able to cooperate in all instances with both CBA and BALB/c B cells, perhaps with a slight bias towards CBA B cells. [Failure to cooperate efficiently with B cells bearing MHC specificities different to those of the thymus of maturation was unlikely to be the result of the 'wrong' haplotype markers on B cells eliciting nonspecific suppressors because in 10 thymic chimaeras tested, cooperation was always observed with (CBA × BALB/c)F$_1$ B cells whatever the restricted specificity of the helper population.]

Table 1 Thymus determines MHC restricted specificities of T-helper cells

Source of thymus graft		Total no. of anti-TNP PFC with the following B-cells.	
		CBA(H–2^k)	BALB/c(H–2^d)
Expt 1*			
C3H/He	Donor 1	1,676	43
	2	1,902	0
BALB/c	Donor 1	367	734
	2	996	3,836
(C3H/He × BALB/c)F$_1$	Donor 1	1,282	1,823
	2	1,784	1,068
No T cells		83	52
Expt 2*			
C3H/He	Donor 3	315	0
BALB/c	3	77	1,570
No T cells		120	97
Expt 3			
C3H/He	Donor 4	2,337	205
	5	2,447	207
BALB/c	Donor 4	1,121	1,943
	5	430	419
(C3H/He × BALB/c)F$_1$	Donor 3	1,804	580
No T cells		567	30
Expt 4			
C3H/He	Donor 6	1,742	320
	7	4,346	321
BALB/c	Donor 6	789	1,681
(C3H/He × BALB/c)F$_1$	Donor 4	2,047	1,646
No T cells		324	437

Thymic chimaeras were constructed as follows:- (C3H/He × BALB/c)F$_1$ mice were thymectomised at 6 weeks of age. One month after thymectomy the mice were irradiated with 875 R and reconstituted with (C3H/He × BALB/c)F$_1$ bone marrow (approximately 5×10^6 bone-marrow cells per recipient) to produce relatively T cell-free (AT × BM) mice. Fifteen days later thymus lobes were obtained from newborn mice, irradiated *in vitro* with 600 R and transplanted under the kidney capsule of each AT × BM recipient, one thymus lobe each. This procedure was designed to provide an environment for the maturation of host T cells without allowing any maturation of thymus-graft donor cells. Seven weeks later all recipients were primed with 250 μg keyhole limpet haemocyanin (KLH) in Freund's complete adjuvant (FCA); after a further 3 weeks all mice were boosted with 100 μg of soluble KLH and after 8–10 days the animals were killed and their spleens taken as a source of T-helper cells for *in vitro* experiments. T-helper function was assessed in an *in vitro* microculture system as previously described[5,8]. In brief, T cells were purified by passage of spleen cells through nylon wool. Red cells were removed by treatment with ammonium chloride and the remaining (T) cells treated at 37°C for 20 min with 25 μg ml^{-1} mitomycin C to eliminate the possibility of antibody production by any residual B cells. 'B cells' were spleen cells from TNP-OVA primed CBA or BALB/c mice, and were treated with anti-Thy 1.2 serum and complement. Helper T cells were incubated for 5 days with both BALB/c and CBA B cells in the presence of antigen (0.1–0.3 μgml^{-1} TNP–KLH). Total direct and indirect PFC to TNP coupled donkey erythrocytes were then determined from 28–30 microculture wells.

* The efficiencies of responses of BALB/c and CBA B-cells were compared in expts 1 and 2 by the addition of primed T cells of pools of (C3H/He × BALB/c)F$_1$ mice. In expt 1 BALB/c B cells gave 2,818 PFC (in 30 wells) while CBA B cells gave 2,215 PFC. In expt 2 BALB/c B cells gave 2,469 PFC while CBA B cells gave 1,831 PFC.

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It is interesting that (CBA × BALB/c)F_1 T cells which had matured in a BALB/c thymus should in most cases have cooperated with CBA B cells to some extent. This 'noise' in such MHC restriction experiments is not uncommon in our hands and was repeatedly observed in situations where BALB/c T cells, whatever the source, have been tested for helper function with CBA B cells. As suggested previously[8] this may represent an interesting one-way cross reaction between BALB/c and CBA MHC associated specificities.

This result is fully compatible with and clarifies previous studies with bone marrow chimaeric mice. Thus: (1) Totally allogeneic ($A \rightarrow B$) chimaeras make poor antibody responses to thymus dependent antigens[12]. In this case A T cells develop in a B thymus and thus cooperate inefficiently with A B cells. (2) T cells from $(A \times B)F_1 \rightarrow A$ chimaeras cooperate with A B cells only[8,9], for they will have matured in an A thymus. (3) T cells from $A + B \rightarrow (A \times B)F_1$ double chimaeras cooperate with both A and B B cells[3,4] for they have developed in an $(A \times B)F_1$ thymus. (4) T cells from $A \rightarrow (A \times B)F_1$ single chimaeras have the potential for cooperation with B B cells[8], again as a result of developing in an $(A \times B)F_1$ thymus.

It is also of interest that in the helper T-cell system it is the I region[2] which determines the observed MHC restrictions, for it is this region which also codes for MHC-linked Ir genes. It is possible to think that MHC restriction and MHC-linked Ir genes are but two faces of the same coin[13], and indeed recent evidence lends support to this view, in studies where it was shown that MHC gene products (presumably in the thymus) can determine the T-cell repertoire to extrinsic (nominal) antigens[14].

These results with helper T cells fulfil what could be predicted from the original findings of Zinkernagel *et al.*[7] with cytotoxic T cells; and reemphasise the point that MHC gene products are involved in the process of differentiation within the thymus.

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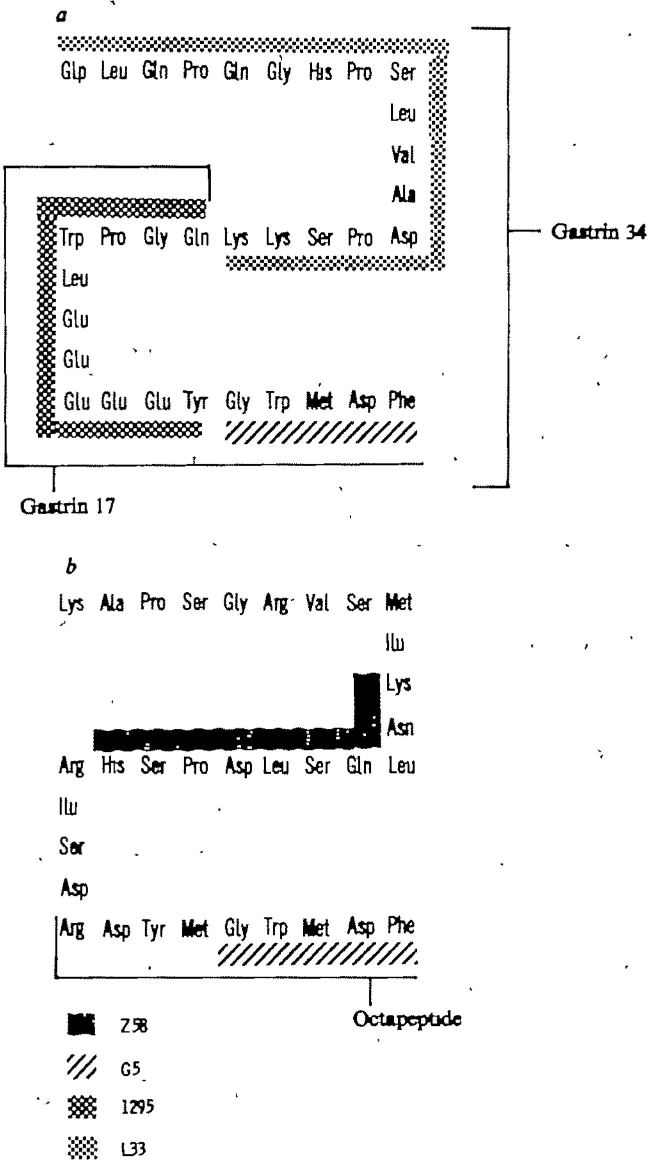
Localisation of intestinal gastrin in a distinct endocrine cell type

GASTRIN extracted from human intestine has two main molecular forms, little (G17) and big (G34) gastrins[1]. Both these peptides are also found in antral tissue although their relative proportions differ, G17 being the major component in antral tissue and G34 that in intestinal tissue[1]. The endocrine cell associated with the production of the immunoreactive gastrin found in the antral tissue is the ultrastructurally classified G cell[2]. The cellular origin of the immunoreactive gastrin extracted from the intestinal tissue has not been determined. The presence of cholecystokinin (CCK) peptides[3,4], closely related to gastrin and sharing with it a C-terminal pentapeptide sequence (see Fig. 1), makes the specific immunological detection of both gastrin and CCK dependent on the use of well characterised and highly specific antibodies. We report here the finding that intestinal gastrin originates in a type of cell totally distinct from the I cell which has recently been identified as the source of CCK[5].

To provide conclusive evidence we have used the semi-thin/thin sectioning method[6] with four different antisera. Each antiserum was specific for a different region of the peptide molecules, as shown in Fig. 1. G5 is a C-terminal-directed antiserum capable of detecting both gastrin and CCK[7]. 1295 Is directed to the N-terminal region of G17 and detects principally little gastrin[8]. L33 is directed to the N-terminal region of big gastrin, G34, and detects the G34 form of gastrin[9]. Z58 was raised to a synthetic peptide with the 9–20 amino acid sequence of CCK; the antibodies detect only CCK from the series of homologous peptides (see Fig. 1). None of the antisera showed significant binding to any other available gut peptide, including secretin, vasoactive intestinal polypeptide, gastric inhibitory polypeptide, neurotensin, motilin, pancreatic polypeptide, somatostatin, glucagon, substance P and bombesin.

Semi-thin (800 nm) sections of human antrum, duodenum and jejunum, fixed in purified glutaraldehyde and embedded in resin, were stained by the indirect immunofluorescence

Fig. 1 Diagrams showing the region of the peptides detected by the antisera used in the study. *a*, Gastrin; *b* CCK.


a
Glp Leu Gln Pro Gln Gly His Pro Ser
Leu
Val
Ala
Trp Pro Gly Gln Lys Lys Ser Pro Asp
Leu
Glu
Glu
Glu Glu Glu Tyr Gly Trp Met Asp Phe
Gastrin 34
Gastrin 17
b
Lys Ala Pro Ser Gly Arg Val Ser Met
Ilu
Lys
Asn
Arg His Ser Pro Asp Leu Ser Gln Leu
Ilu
Ser
Asp
Arg Asp Tyr Met Gly Trp Met Asp Phe
Octapeptide
Z58
G5
1295
L33

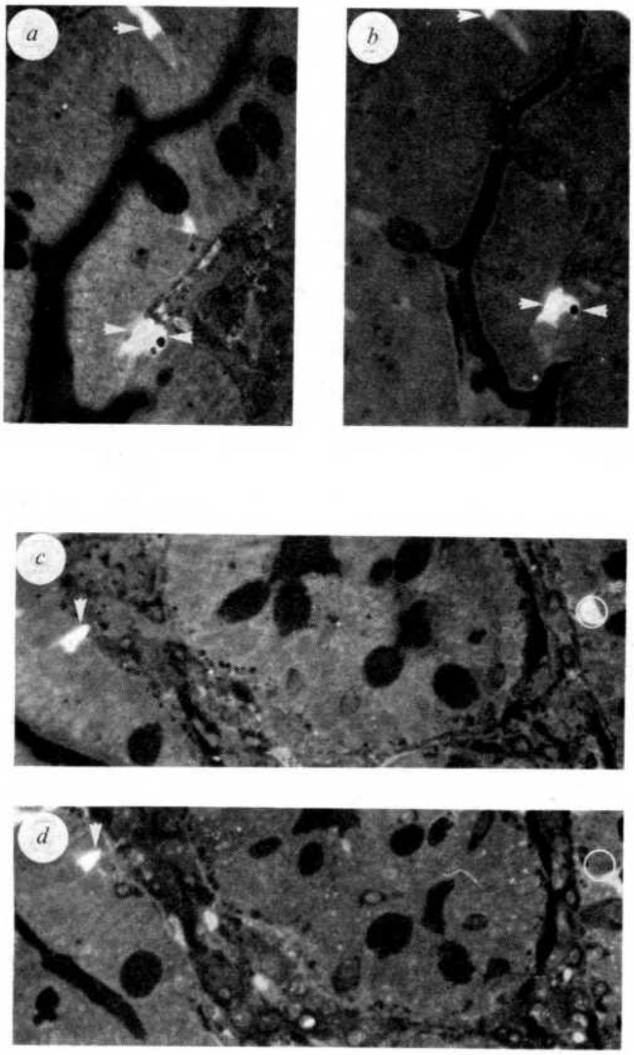


Fig. 2 800-nm (semi-thin) sections of human duodenum fixed in 2.5% purified glutaraldehyde. The araldite was removed by immersion in saturated NaOH in ethanol. The sections were stained by the indirect immunofluorescent technique using four region-specific antisera. *a*, Section stained with the 1295, N-terminal G17-specific antiserum, 1:300, for 24 h at 4 °C. Shows two positive cells, indicated by the arrows. ×1,500. *b*, Serial section to *a* stained with the L33, N-terminal G34-specific antiserum, 1:100, for 24 h at 4 °C. Shows the same three positive cells. ×1,500. *c*, Section stained with G5, the C-terminal gastrin/CCK antiserum, showing two positive cells made up of two separate populations: gastrin cells, marked by a circle; CCK cells, marked by an arrow (see *d*). ×1,500. *d*, Serial section to *c* stained with Z58, the CCK-specific antiserum, showing the same arrowed cell as in *c*, positive, while the circled cell is negative. ×1,500.

method[10]. Serial sections were incubated with 1295 (1:300), G5 (1:100), L33 (1:100) and Z58 (1:200) overnight at 4 °C. A second layer of fluoresceinisothiocyanate (FITC)-conjugated antirabbit globulin was applied for 1 h at room temperature. The specificity of the immunostaining was established by previous incubation with purified peptides as detailed in Table 1. The results of the tissue controls confirm the data already available on the specificity of the antisera. Additional controls were the incubation of serial semi-thin sections with normal rabbit serum (1:100) and FITC-conjugate alone, neither of which showed any positive cells.

The results of the immunostaining on the semi-thin (800 nm) sections of antral tissue showed that L33, 1295 and G5 all stained identical cells. These cells were then identified on the serial thin (60 nm) sections by means of easily located landmarks, such as mucin cells, which are visible in both semi-thin and thin sections. The ultrastructural appearance of the cells identified in this way was that of typical G cells with secretory granules ranging from large (350 nm) and electronlucent to small (190 nm) and electron dense. Z58 was negative on antral tissue as expected.

The semi-thin sections of duodenum and jejunum gave the following results. L33 and 1295 stained identical cells (Fig. 2*a*, *b*), Z58 stained a completely different population of cells (Fig. 2*d*), and G5 stained both cell populations (Fig. 2*c*).

As Z58 is specific for CCK and G5 is capable of binding to both CCK and gastrin, we conclude that the cells stained by both these antisera correspond to the I cells already characterised as the source of CCK[5]. This was confirmed by the appearance of the cells at an ultrastructural level. The cells stained by L33, 1295 and G5 must, by virtue of the specificity of the L33 and 1295 antisera, contain the immunoreactive gastrin present in the intestine. On serial thin sections these cells were found to be of a single type, with secretory granules which were small, round and electron dense (Fig. 3*a*–*c*). Electron micrographs of these cells were used to measure the secretory granules; 650 granules from 12 cells were measured, giving a mean diameter of 175 ± 24 nm (mean ± s.d.). When the measurements were corrected for sectioning artefact[11], the final value for the mean diameter was 220 ± 20 nm.

Clearly, the cells containing gastrin in the intestine are of a different morphological type from the G cells of the antral mucosa. They totally lack the large electronlucent granules that are so characteristic of the antral (GA) cells. Recent work has shown that the antral mucosa contains equimolar amounts of G17 and the N-terminal fragment left after cleavage of G34 to G17 (ref. 9), in addition to smaller quantities of G34 itself. It is possible that the large electronlucent granules in the antral (GA) cells contain the products of the enzymatic cleavage of G34, whereas the small granules present in the same cells contain mainly unaltered G34. Thus, the granules of the intestinal gastrin (GI) cells could correspond to these small G-cell granules

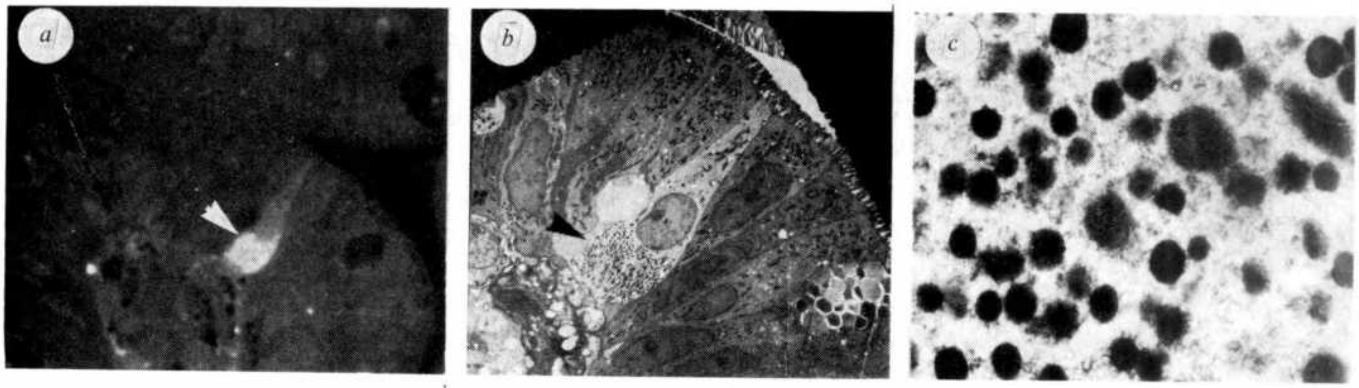


Fig. 3 *a*, 800-nm section prepared as described in Fig. 2, stained with 1295 (1:300), the N-terminal G17 antiserum. The arrow indicates the positive endocrine cell. ×1,500. *b*, Serial thin (60 nm) section showing the same endocrine cell. Note the connection to the gut lumen by microvilli and the grouping of the secretory granules close to the basement membrane. ×2,000. *c*, High magnification electronmicrograph of the endocrine cell to show details of the secretory granules. ×27,000.

Table 1 Specificity of immunostaining

Antisera (dilution)	Absorption	Peptides (nM) Gastrin 17	Gastrin 34	CCK octapeptide	CCK 1–33	Area of gut
G5 (1:100)	Amount	0 3	ND	1	2	Antrum Duodenum Jejunum
	Result	—		—	—	
1295 (1:300)	Amount	1	5	10	10	Antrum Duodenum Jejunum
	Result	—	+++	+++	+++	
L33 (1.100)	Amount	10	2.5	10	10	Antrum Duodenum Jejunum
	Result	+++	—	+++	+++	
Z58 (1:200)	Amount	10	ND	10	1	Duodenum Jejunum
	Result	+++		+++	—	

All absorptions were carried out by pre-incubation of the diluted antisera with the peptides overnight at 4 °C before staining the tissue. +++, Positive immunostaining, –, no immunostaining; ND, not done; in this case the gastrin 34 peptide was only available in extremely small quantities, therefore only essential absorptions were undertaken.

and these may also contain mainly unaltered G34, which is the predominant molecular form of gastrin in the intestine.

Information from the study of tissue cultures carried out on gastrinoma cells[12] can be interpreted in the light of these findings. The cells in the original tumour contained the characteristic large electronlucent granules of G-cell type and the majority of the extracted gastrin was G17. After 2 weeks of culture the secretory granules had become progressively smaller and the content of the culture was predominantly G34.

The cells which we have identified as the origin of intestinal gastrin have probably been included in previous classifications as belonging to the ubiquitous D_1 cell category. These cells have been defined as containing small (140–190 nm) round granules, with moderate osmiophilia and a closely applied membrane. The retention of the D_1 nomenclature for the duodenal/jejunal cells containing gastrin would be confusing due to the widespread distribution of cells corresponding to the above description[13]. As the new variety of gastrin-containing cells is confined to the intestinal mucosa, we propose the name GI for these cells, denoting their intestinal origin, as distinct from the GA cells which are mostly confined to the antral region of the stomach. This is in keeping with the move towards a functional rather than morphological classification initiated in the Lausanne revision of the original Wiesbaden (1969) endocrine cell classification. Two new cell types have now been named after their peptide product, the N or neurotensin-containing cell[13] and the PP or pancreatic polypeptide-containing cell[13].

In previous classifications a functional emphasis was not possible because there was insufficient evidence about the peptide products of the various cell types. The widely accepted semi-thin/thin technique, in combination with technical advances in antibody production and improved immunocytochemical methods have made a functional classification possible.

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Identification of the neuritogen for experimental allergic neuritis

EXPERIMENTAL ALLERGIC NEURITIS (EAN) is a model of human acute inflammatory polyneuropathy, Guillain–Barré syndrome. It can be produced in several species of laboratory animal by immunisation with homologous or heterologous peripheral nerve emulsified in Freund's complete adjuvant[1,2]. After about two weeks the animals may develop signs varying from ataxia to paralysis and death. EAN has been produced using crude acid extracts of peripheral nerve[3,4], but investigations into the neuritogenic properties of the component proteins of peripheral nerve myelin have yielded inconclusive results[5–10]. In experimental allergic encephalomyelitis (EAE), the central nervous system (CNS) counterpart of EAN, the encephalitogenic antigen has been shown to be a CNS myelin basic protein of molecular weight 18,000 (ref. 11). The three major proteins of peripheral nerve myelin, which together account for about 75% of the myelin protein, are usually referred to as P_0, P_1 and P_2 (ref. 12). P_0 is a glycoprotein of molecular weight 30,000 and P_1 and P_2 are both basic proteins with molecular weights of 18,000 and 12,000–14,000, respectively[12–13]. We describe here a reliable method for producing severe EAN in inbred Lewis rats with bovine intradural root material, and show that intact P_2, the major basic protein of peripheral nerve myelin, is the responsible neuritogen.

Bovine intradural root was obtained within 30 min of death and myelin was prepared from this by sucrose density centrifugation[15]. The crude acid extract was prepared by incubating myelin in 30 mM HCl for 16 h and collecting the supernatant

Table 1 Production of EAN in Lewis rats with whole myelin and pure P_2 from bovine intradural root

					Histological disease		
					EAN		EAE
	Dose	Day of	Clinical disease				
Antigen	(mg per animal)	onset	Incidence	Mean score	Incidence	Mean score	Incidence
Saline	—	—	0/2	—	0/2	—	0/2
FCA	—	—	0/3	—	0/3	—	0/3
Myelin (5)	2.5	12.0	25/25	2.5	24/24	2.6	0/24
Acid extract (2)	0.25	12.9	8/9	2.0	9/9	2.4	0/9
P_2 (2)	0 25	12.5	10/10	2.1	10/10	2.6	0/10

All samples except saline and adjuvant controls were emulsified in an equal volume of Freund's complete adjuvant (FCA) containing 10 mg ml^{-1} *Mycobacterium tuberculosis* H37RA (Difco). The numbers in parentheses refer to the number of individual experiments carried out; a different antigen preparation was used for each. For scoring the clinical disease, the animals were assessed daily after day 7 according to the scale: 0 = normal; 1 = limp tail; 2 = mild or moderate paraparesis; 3 = severe paraparesis, tetraparesis or paraplegia. Scoring of histological disease were. 0 = normal or equivocal lesions, 1 = mild, 2 = moderate and 3 = severe, invasion of inflammatory cells.

after centrifugation. The basic protein, P_2, was purified from this crude extract by ion exchange[7] and gel exclusion[16] chromatography. All steps were carried out at 4 °C in the presence of 1 mM mercaptoethanol; 5 g of lyophilised myelin yielded approximately 30 mg of P_2. This was judged to be pure by the presence of a single band on SDS-polyacrylamide electrophoresis, similarity of amino acid content to that in previous reports (especially the absence of histidine)[13,14], and the absence of ganglioside.

Male inbred Lewis rats (175–225 g) were given two subcutaneous injections of 50 μl emulsion, one just proximal to each fore-footpad, and were weighed and examined daily. All remaining animals were killed on day 21. Longitudinal sections of each sciatic nerve, the cervical and thoracic cord and brain, and six transverse sections of the lumbosacral cord and cauda equina from each animal were stained with haematoxylin and eosin and examined by light microscopy.

Preliminary experiments revealed bovine intradural root to be the best starting material and the inbred Lewis rat to be the most convenient species. In five separate experiments (Table 1), each with a different preparation of bovine intradural root myelin, moderate or severe clinical and histological EAN was always produced and the CNS showed no evidence of cellular infiltration. The lack of CNS involvement when using bovine peripheral myelin agrees with a recent report[17]. Clinical signs first appeared in some animals on day 11, with a mean onset of 12.0 d. This was accompanied by a weight loss to 75% of the starting weight.

The acid extract was then shown to be neuritogenic at a dose (0.25 mg per animal) which was calculated to contain a similar amount of P_2 as 2.5 mg whole myelin[12]. Finally, pure P_2 was shown to be neuritogenic at a dose of 0.25 mg. No CNS involvement was found in any of the animals treated with either the acid extract or pure P_2.

Of the two basic proteins in peripheral myelin, P_1 has been shown to be the same as the encephalitogenic basic protein in the rabbit sciatic nerve[18], and this is likely to be the same for other species. Thus, attention has focused on the other basic protein, P_2, which may be specific to peripheral nerve myelin[13]. Previous attempts to induce EAN with a pure isolated protein have used bovine intradural root BF (same as P_2) in the guinea pig[6], bovine intradural root P_2 in the rabbit[7] and rabbit sciatic nerve P_2 in the monkey and guinea pig[5]. All but the last were unsuccessful, and this result was later withdrawn[7] with the suggestion that isolation might lead to an alteration in the considerable secondary structure of P_2. In confirmation of this, Brostoff *et al*[10] have reported that a cyanogen bromide peptide of P_2, but not whole P_2, was neuritogenic. In addition, Abramsky *et al.*[8,9] have isolated a basic protein of molecular weight 14,000 from human and bovine sciatic nerves which they reported to be neuritogenic. In the first report[8] this protein, known as P_1L, was claimed to differ from all known myelin proteins, whereas in the latter[9] it was thought to be the same as P_2. In both reports, however, the disease produced was very mild, with an incidence as low as 50% in some experiments. Moreover, others have suggested that the reported amino acid content of P_1L indicates contamination with other proteins, especially P_1 (ref. 14).

In the present study, a 100% incidence of pure EAN without EAE has been produced by the use of bovine intradural root P_2 in the inbred Lewis rat, a combination not previously reported. Thus, in contrast to the experience of others[10], we have been able to isolate P_2 in a form which is still neuritogenic. Overall, the clinical disease produced by the pure neuritogen was slightly less severe than that produced by whole myelin, but, nevertheless, several animals developed the maximum score on our scale. A possible reason for the lack of success of previous authors, apart from the combination of tissue and recipient, may be their predominant use of the guinea pig[4–9], because this species has been shown to possess very little P_2 in its peripheral myelin[12]. Thus, guinea pigs immunised with peripheral myelin basic proteins have been reported to remain unaffected[6,7], develop EAE[5] or a low incidence of a very mild EAN[9]. In our preliminary investigation with the guinea pig, we found that bovine intradural root produced only EAE and not EAN

Now that we have clearly identified the neuritogen involved in the animal model of Guillain–Barré syndrome, we intend to extend our study to look for evidence of sensitisation to P_2 in the human disease.

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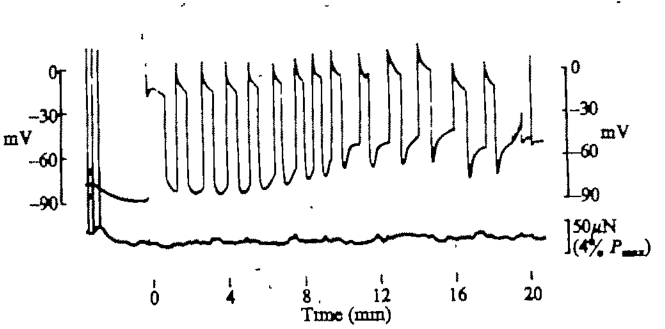
Are cardiac muscle cells 'skinned' by EGTA or EDTA?

KNOWLEDGE of the processes involved in the contraction of skeletal muscle fibres has been greatly advanced by study of the 'skinned' fibre preparation. Here the sarcolemma is peeled off, giving access to the intracellular organelles unhampered by an intervening cell membrane. The small size of cardiac muscle cells had seemed to preclude the use of this technique, and so interest has been shown in a 'chemical' method for 'skinning' heart cells, which involves exposing the muscle to EGTA and EDTA (ref. 1). However, this paper questions the effectiveness of the EDTA/EGTA skinning methods, reporting that tension production in fully treated cells is $[\mathrm{Na}]_o$ dependent, and that the cells can support resting and action potentials in low $[\mathrm{K}]_o$-'skinning' media. It is concluded that both the high Ca-sensitivity and low membrane potential of EGTA/EDTA-treated cells occur without radical impairment of the sarcolemmal permeability barrier. An alternative explanation for the high sensitivity to extracellular calcium is presented which involves the Ca–Na exchange mechanism.

Treating cardiac muscle with divalent cation chelators such as EDTA and EGTA is reported to produce a 'high permeability state' after 12–20 min exposure to a medium which typically includes KCl, ATP and a buffer, in addition to the chelator[1–6]. The evidence that this procedure makes the heart cell membrane 'highly permeable to the calcium buffer system' (ref. 1) depends heavily on two principle observations: first, the treated cells have a very low resting potential, suggesting that the sarcolemma has become freely permeable to small ions[1,4,5], and second, that tension can be induced by Ca^{2+} at micromolar levels. Mechanical activity at these low concentrations is normally associated with the activation of isolated contractile proteins or of skeletal fibres skinned by more direct methods[1–6]. The EDTA/EGTA method, or a variation of it, has subsequently been applied to several cardiac tissues, smooth muscle[7] and skeletal muscle[8]. However, recent studies of frog heart[9,10] have suggested that the two above characteristics are not unique to skinned cells and, therefore, are not unequivocally indicative of 'skinning'.

In these previous experiments, and those to be reported here, EGTA- and EDTA-containing solutions have been applied to fine atrial and ventricular trabeculae of frog heart by rapid superfusion. The results may be summarised thus. First, protracted action potentials are produced in EGTA-Ringer solution; these can arise spontaneously and eventually last up to a few minutes. Unless observed by continuous recording from one cell, this behaviour can give the appearance of a full depolarisation of the cell. However, even in preparations exposed to EGTA for over an hour the action potential can be curtailed by acetylcholine (ACh) or divalent cations[9]. The resting potential between the long action potentials is well maintained and, by inference, the cell membrane is still an effective permeability barrier. The effects also occur with EDTA and are more marked; spontaneous activity is common and action potential prolongation even greater. Figure 1 shows a typical record where the exposure to EDTA was started with the preparation already in the presence of ACh. ACh induces hyperpolarisation. Addition of EDTA rapidly evokes a spontaneous train of long action potentials which is sustained over 15 min. Restoration of Ca^{2+} returns electrical (and eventually mechanical) activity to near normal.

Fig. 1 Electrical activity in EDTA-containing solutions. Membrane potential (upper trace) and tension (lower trace) from a superfused frog ventricle trabeculum (diameter ~200 µm, length 2 mm). On addition of acetylcholine (10^{-5} M) to the Ringer solution (see ref. 9) stimulation was halted. Removing Ca and adding 3 mM EDTA induced spontaneous activity which persisted until EDTA was removed and Ca restored (after 17 min). A stimulus (at 20 min) produced an action potential of near-normal duration. Temperature 20 °C.



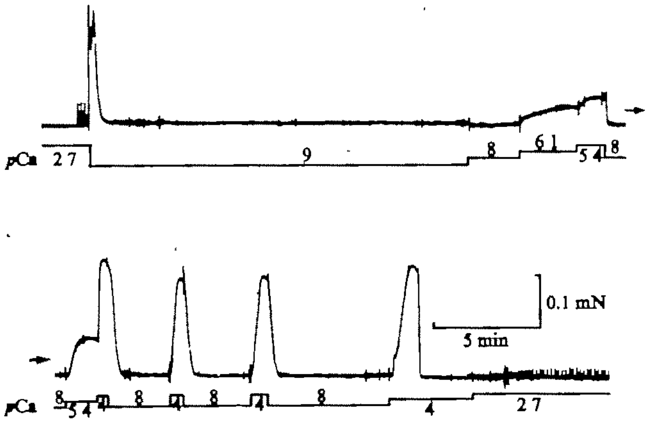


Fig. 2 Tension responses of a superfused frog atrial trabeculum (diameter ~100 µm, length 1.5 mm). Changing from Ringer's solution (pCa 2.7 = 2 mM) to Winegrad's 'disruption' medium (mM; KCl 120, $\mathrm{Na_2ATP}$ 5, Tris 20, EDTA 5, pCa ~9) induced a contracture. pCa values of less than 9 indicate that the 'activating' solution (mM; KCl 120, $\mathrm{Na_2ATP}$ 5, Tris 20, $\mathrm{MgCl_2}$ 1, EGTA 5, Ca 0–5) was used. Halfway through the final exposure to pCa 4, the 'activating' solution was substituted by Ringer's solution at the same pCa value. Temperature 20 °C, pH 7.00

The second and, apparently for most authors it seems, the major criterion of the 'high-permeability state', namely high sensitivity to calcium, can also be displayed by cardiac cells without skinning them. Frog atrial trabeculae exposed to low sodium concentrations (with and without EGTA) produce half-maximal contracture tension at a $[\mathrm{Ca}^{2+}]$ of $\sim 2\times 10^{-7}$ M with a normal resting membrane potential[10]. This finding has been extended to frog ventricle where half-maximum tension in identical conditions occurs at 1.2 (±s.d., 0.5) $\times 10^{-6}$ M Ca^{2+} ($n = 6$). Relaxation is very slow in these conditions ($t_{1/2}$ of the order of 250 s at pCa 6 compared with about 10 s at pCa 4–2)[10,14] and can give the appearance of a tonic rather than a phasic response. Until relaxation is completed, tension steps can be induced by pCa changes.

As both criteria for skinning fail to distinguish clearly between hypothetical skinned cells and those described above which are patently unskinned, it must be asked if cells exposed to the full EDTA and EGTA 'skinning' treatment are really skinned at all.

To identify whether the preparation undergoes a qualitative change in Winegrad's 'disruption' medium, the constituents of this medium were tested in various permutations. In summary, the high permeability state, judged on the development of high Ca sensitivity (repeatable step-wise responses to pCa changes), loss of membrane potential (except obviously in K-rich media) or production of rigor (high Ca, ATP-free media), could not be demonstrated even with the combination of EDTA or EGTA (up to 10 mM), high $[\mathrm{K}^+]$, low $[\mathrm{Na}^+]$ and Tris buffer. However,

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inclusion of ATP, which is present in Winegrad's full disruption medium, did produce a qualitative change in contractile behaviour.

If pCa steps were made after 20 min exposure of a frog ventricle or auricle trabeculum to EDTA or EGTA ($p\text{Ca} \geq 8$, see legend to Fig. 2), then tension steps could be induced in the range of pCa 7–4. However, there are at least four clear indications that these preparations are not skinned. (1) Spontaneous relaxation occurs, although at a very slow rate; (2) peak contracture tension at a given pCa falls with the duration of the exposure to the 'skinning' medium: the highest Ca sensitivity in these preparations develops within a minute but reaches a lower steady level after about 20 min; (3) rapid, full relaxation is produced when Ringer's solution is restored; (4) In an ATP + EGTA (or EDTA) Ringer solution ($p\text{Ca} > 8$), as before, long-lasting ACh-sensitive action potentials can be recorded for over an hour.

The third point is illustrated in Fig. 2. If these cells were in fact skinned, then restoring Ringer's solution (removing MgATP) while the muscle is contracted should, if anything, induce rigor[11]. Similar, though much slower, effects are visible in Winegrad's published records (ref. 1, Fig. 1). He interpreted this as due to a 'healing over' of the cell membrane at the raised calcium level. This argument can be discounted, as no relaxation occurs even at $p\text{Ca} < 2$ (see, for example, ref. 12, Figs 1, 2, 4) and relaxation occurs even without altering pCa (Fig. 2).

The relaxation produced by restoring Ringer's solution is primarily the result of raising the sodium concentration. This was shown by investigating the effect of $[\text{Na}^+]$ on contracture strength in preparations that would otherwise be judged 'highly permeable'. Figure 3 shows the effect of $[\text{Na}^+]$ from 6.6 to 63 mM on contracture strength at constant pCa. Three typical tension reponses are shown on the right, from which it is clear that peak force is reduced as sodium concentration increases (note that $[\text{K}^+]$ is constant and tonicity maintained with Tris). Results from one preparation are plotted on the left. The steep dependence of contractile strength at constant pCa on $[\text{Na}^+]$ is not characteristic of skinned muscle but is reminiscent of responses to simple low-sodium media (ref. 10, Fig. 4). An expression derived in that paper (Eq. A6) relates contracture strength to $[\text{Na}^+]_o$ at constant $[\text{Ca}^{2+}]$, and by choosing a suitable value for $K_{\text{Na}}^{\text{app}}$ (which alters the position but not the shape of the curve) a good fit is obtained. The curve is shifted to the right on the Na axis at lower pCa values. This expression relates to the behaviour of a Na–Ca exchange system in the sarcolemma of frog heart. The results indicate that these supposedly skinned cells are not skinned at all, but rather that the tension reponses observed result from the operation of the Ca–Na exchange system whose activity has been modified by depolarisation (120 mM K^+) and perhaps more importantly, by extracellular ATP.

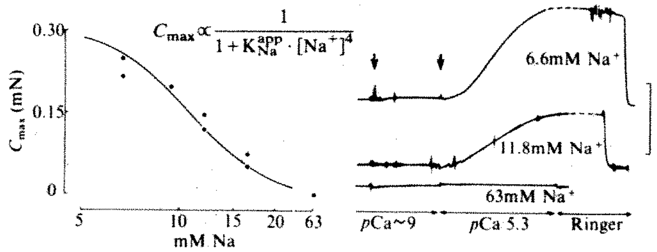

Fig. 3 Right: tension records obtained after a 2-min exposure to the 'disruption' solution (see Fig. 2), at which time Ca-sensitivity is maximal. At the first arrow, the 'activating' solution was applied (pCa ~8, Na_2ATP 3.3 mM and Tris 127 mM otherwise as in Fig. 2, that is, hypertonic). At the second arrow, 1 min later, the pCa was reduced to 5.3 with $[\text{Na}]_o$ varied by substituting NaCl for Tris-Cl. Finally, normal Ringer's was restored. Left: results from one experiment of the type shown on the right relating peak contracture tension ($C_{\max}$) to $[\text{Na}^+]_o$. The curve was drawn according to the equation and scaled to maximum possible tension taken as that observed in a 120 mM KCl, 0 mM NaCl solution[11]. The value of $K_{\text{Na}}^{\text{app}}$ in this case was $1.34 \times 10^8\ \text{M}^{-4}$. Calibration bar = 0.15 mN, temperature 20 °C, pH 7.00. (*Rana esculenta.*)

Frog heart is not rendered 'highly permeable' by Winegrad's method. Whether a similar criticism may be levelled at results obtained with other cardiac and non-cardiac tissues using this technique[2,4,7,8] is open to speculation, although morphological studies of human skeletal muscle are reported to have shown disruption of the sarcolemma[8]. Long-lasting action potentials in EDTA have been reported for mammalian cardiac tissue[13]. The results reported here clearly indicate that the two properties of high calcium sensitivity and low resting potential are not sufficient indices of successful skinning. It seems that only the elegant and painstaking microdissection techniques of Fabiato and Fabiato[15] ensure that a cardiac cell can be skinned while leaving the sarcoplasmic reticulum and mitochondria intact.

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Activation of adenylate cyclase by vanadate

VANADATE has been identified as a potent inhibitor of (Na,K)ATPases[1,2]. The (Na,K)ATPase of dog kidney is inhibited 50% by 40 nM sodium orthovanadate (Na_2VO_4) in optimal conditions (28 mM Mg^{2+})[2]. As vanadate was originally identified as an inhibitor in muscle-derived ATP preparations[2] and in equine and rabbit skeletal muscles, it has been suggested as endogenous regulator of (Na,K)ATPase[2–4]. We have tried to use vanadate as a tool to inhibit ATPases in adenylate cyclase studies when working at low ATP concentrations. We report here that vanadate causes marked stimulation of adenylate cyclase activity in membranes isolated from rat fat cells and produces maximal activation even in the absence of hormones.

Plasma membranes of isolated fat cells were prepared from epididymal adipose tissue of male Sprague-Dawley rats (160–220 g). The preparation followed essentially the method of McKeel and Jarett[5]. The determination of adenylate cyclase was carried out as described previously[6] using the protein-binding method of Gilman[7]. The assay contained in a total volume of 100 µl: 1 mM ATP, 20 mM MgCl_2, 40 mM Tris-HCl, pH 7.4, 5 mM creatine phosphate, 10 µg creatine kinase (25 units per mg) and 20 µg bovine serum albumin. The reaction mixture was preincubated for 5 min at 37 °C and the reaction started by addition of freshly thawed plasma membranes (4–8 µg per test tube). For blank values, heat-inactivated membranes were added to the reaction mixture. The reaction was stopped after 10 min incubation at 37 °C by addition of 1 ml of ice-cold

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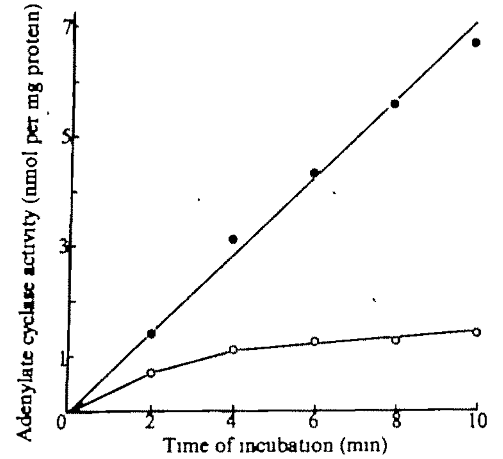

Fig. 1 Time course of adenylate cyclase activation by vanadate. Purified plasma membranes were isolated from rat fat cells by a previously described method[5]. Fat cell membranes (4.4 μg of membrane protein per 100 μl) were incubated at 37 °C in a medium containing 1 mM ATP with an ATP-regenerating system, 20 mM $MgCl_2$, 0.2% bovine serum albumin and 40 mM Tris-HCl buffer, pH 7.4. Activity was assayed without (○) and with 1 mM sodium vanadate (●). Values are means of triplicate determinations.

50 mM sodium acetate buffer, pH 4.0. Cyclic AMP was determined after 60-fold dilution of the samples. In these conditions, vanadate up to a concentration of 1 mM did not interfere with the assay procedure. Compounds used were anhydrous sodium vanadate ($NaVO_3$) and sodium fluoride (both Merck Darmstadt), theophylline (Boehringer Ingelheim), 1-noradrenaline-HCl (Fluka), guanylyl imidodiphosphate (GppNHp) and ATP (both Boehringer Mannheim).

Figure 1 shows a typical effect of sodium vanadate on the time course of adenylate cyclase activity in purified plasma membranes from rat fat cells. At a concentration of 1 mM, vanadate elicited an immediate stimulatory effect on enzyme activity which was linear up to 10 min. The increase of basal activity by vanadate was twofold after 2 min and more than fourfold after 10 min of incubation, the difference being due to a nonlinear time course of basal activity.

As can be seen from Fig. 2, the stimulatory effect of sodium vanadate was concentration dependent. A significant increase of cyclase activity was observed even with 10 μM vanadate. Similar dose–response curves were obtained with ammonium vanadate (NH_4VO_3). Concentrations higher than 1 mM vanadate have not been tested.

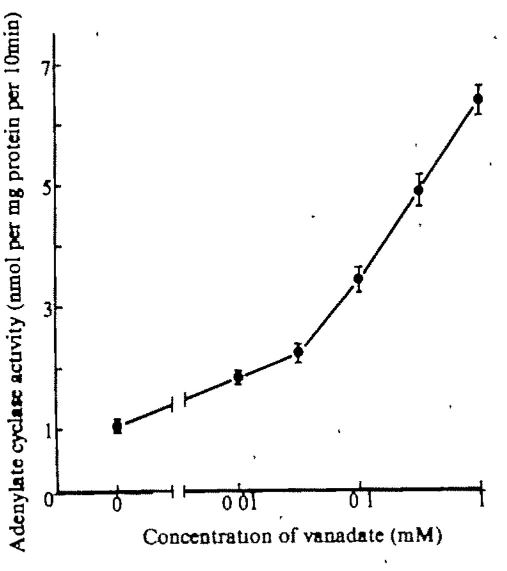

Fig. 2 Concentration dependence of vanadate stimulation of fat cell adenylate cyclase activity. Fat cell membranes were incubated as described in the legend to Fig. 1. Values are means ± s.e.m. of triplicate determinations.

The effect of vanadate on adenylate cyclase activity was further studied in the presence of a phosphodiesterase inhibitor and various stimulators of adenylate cyclase (Table 1). In the presence of theophylline, which itself had only a marginal effect on basal activity, the stimulatory effect of vanadate remained unchanged. Thus, it seems unlikely that vanadate might act as a phosphodiesterase inhibitor of a residual phosphodiesterase activity. Furthermore, vanadate in concentrations up to 1 mM had no effect on the activity of soluble and particulate cyclic AMP phosphodiesterases of rat fat cells (data not shown). The effect of 10 μM noradrenaline was further increased by addition of vanadate, but the effects of the two agents were not additive. The activity obtained with NaF plus vanadate was somewhat lower than that with NaF alone. Vanadate increased the effect of a low concentration of GppNHp (0.1 μM), but had no additional effect on a higher GppNHp concentration (1 μM).

As it is a potent inhibitor of (Na,K)ATPase activity[1,2], it seemed possible that vanadate might increase cyclase activity by preserving ATP as substrate for adenylate cyclase. However, cyclase activation by vanadate was independent of the concentration of ATP, tested in the range 1 μM–1 mM (data not shown). In addition, the ATP-regenerating system used in the present study effectively preserved the ATP concentration even at concentrations lower than 1 mM (ref. 6).

Table 1 Effect of vanadate on adenylate cyclase activity of rat fat cell membranes in the presence of theophylline or various stimulators of adenylate cyclase

Additions	Control	1 mM vanadate
None	1.04±0.04	6.43±0.25
Theophylline, 1 mM	1.20±0.01	6.38±0.22
Noradrenaline, 10 μM	2.05±0.03	6.77±0.49
NaF, 3 mM	13.28±0.76	11.89±0.10
GppNHp, 0 1 μM	3.05±0.24	5.89±0.18
GppNHp, 1 μM	7.95±0.16	8.13±0.10

Fat cell membranes (4 4 μg of membrane protein per 100 μl) were incubated for 10 min at 37 °C in a medium containing 1 mM ATP with an ATP-regenerating system, 20 mM $MgCl_2$, 0.2% bovine serum albumin and 40 mM Tris-HCl buffer, pH 7.4. Each experiment was run in triplicate, values are means±s.e.m.

Another hypothetical explanation for a mechanism of action of vanadate might be that in addition to its inhibitory effect on (Na,K)ATPases, it may affect other nucleoside triphosphatases and cause activation of adenylate cyclase by inhibiting a GTPase associated with the guanylnucleotide binding protein. It has been suggested that the guanylnucleotide binding protein of the adenylate cyclase system plays a dual role[8]; in the presence of hormone plus GTP it activates the adenylate cyclase, but also functions in the hydrolysis of bound GTP and thus terminates the activation. The latter reaction has been referred to as the turn-off GTPase reaction[8]. Any decrease in the rate of hydrolysis of GTP at the regulatory site would maintain more of the enzyme at the active state. A reduction of GTP hydrolysis has been proposed as the basis for increased adenylate cyclase activity after cholera toxin treatment[9]. Furthermore an inhibition of catecholamine-stimulated GTPases by cholera toxin and an enhancement of GTP-dependent adenylate cyclase activity have recently been observed in turkey erythrocyte membranes[8]. Experiments are in progress to determine whether the adenylate cyclase activity elicited by vanadate is GTP dependent.

The effect of vanadate on cyclic AMP formation in addition to its effect on (Na,K)ATPases may be of general interest, as vanadate exerts biological activities in several intact cellular systems. A positive inotropic effect of vanadate in electrically driven cat papillary muscles[10], and several pharmacological effects, among them a powerful peripheral vasoconstriction, have been reported[11].

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Antibody against a stimulatory factor of RNA polymerase II inhibits nuclear RNA synthesis

THE mechanisms by which eukaryotic genes are differentially activated are mostly unknown. However, the most fundamental process of gene expression is thought to be transcription by RNA polymerase II, and thus the regulation of RNA synthesis by this enzyme is one of the important points of study of eukaryotic gene expression. It is generally believed that structural modification of chromatin governs the transcriptability of the template[1], and consequently studies have been carried out on the activity of chromatin[2–4]. Recently, another possibility has been considered, that various regulatory proteins may be involved in the process of transcription and regulate the gene expression[5]. However, although there are many reports of proteins stimulating the activity of RNA polymerase II *in vitro*[6–11], no conclusive evidence has yet been obtained to show that these proteins are, in fact, functioning in the process of nuclear RNA synthesis. We have purified a protein from Ehrlich ascites tumour cells that specifically stimulates the activity of RNA polymerase II on homologous DNA[12]. Named S-II, this is a basic protein with a molecular weight of 40,500. We report here that an antibody against S-II markedly inhibits RNA synthesis in isolated nuclei, but that it does not affect the activity of purified RNA polymerase II. These findings suggest that the processes of RNA synthesis in isolated nuclei and with purified RNA polymerase II and deproteinised DNA are basically different, and that S-II has an essential role in nuclear RNA synthesis.

Protein factor S-II was extensively purified from Ehrlich ascites tumour cells, and antibody against S-II was prepared by injecting S-II into albino rabbits. This antibody was found to inhibit the stimulatory activity of S-II specifically. Nuclei were prepared from Ehrlich ascites tumour cells by the method of Marzluff *et al.*[13], and were active in the incorporation of ^{3}H-UTP into RNA *in vitro*, in the presence of three other nucleoside triphosphates and salts.

First, we tested whether the antibody against S-II had any effect on RNA synthesis in the isolated nuclei. There are three species of RNA polymerase (I–III) in nuclei; RNA polymerases I and III, which synthesise ribosomal RNA and small molecular weight RNA, respectively, are fairly resistant to α-amanitin, whereas RNA polymerase II, which synthesises messenger RNA, is inhibited by α-amanitin[14]. Therefore, in tests on the effects of the antibody against S-II on the activities of these three enzymes, assays were carried out in the presence and absence of 5 μg ml^{-1} of α-amanitin. Nuclei were preincubated in the reaction mixture for RNA synthesis (see legend to Fig. 1) for 30 min at 0 °C with increasing amounts of antibody; the incubation temperature was then shifted to 25 °C and the incorporation of ^{3}H-UTP into the acid-insoluble fraction in 10 min was measured. The control contained immunoglobulin G prepared from non-immunised rabbit serum instead of antibody. As shown in Fig. 1*a*, the antibody inhibited nearly 50% of the α-amanitin-sensitive RNA synthesis, whereas normal IgG had little effect. Neither antibody nor normal IgG affected RNA synthesis in the presence of α-amanitin (Fig. 1*b*). These results clearly indicate that stimulatory factor S-II must be involved in some way in the RNA synthesis catalysed by RNA polymerase II in isolated nuclei, but that it is not related to α-amanitin-resistant RNA synthesis. The results are consistent with our previous findings with isolated enzymes that S-II specifically stimulated RNA polymerase II and did not stimulate RNA polymerase I at all.

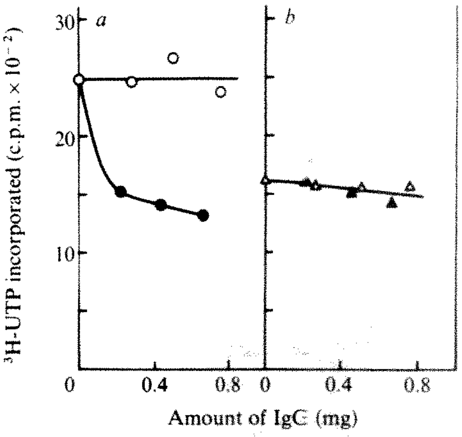

Fig. 1 Inhibition of nuclear RNA synthesis *in vitro* by antibody against S-II. The reaction mixture contained, in a total volume of 100 μl, 50 mM HEPES, *p*H 8.0, 0.25 mM each of ATP, GTP and CTP, 0.025 mM UTP, 2 μCi of ^{3}H-UTP (20 Ci mmol^{-1}), 1 mM $MnCl_2$, 5 mM $Mg(OAc)_2$, 150 mM KCl, 12 mM β-mercaptoethanol, 0.05% Triton X-100, 10% glycerol, 0.5 μg of α-amanitin when present, 2×10^5 nuclei and the indicated amount of antibody against S-II or normal IgG. The mixture was preincubated for 30 min at 0 °C, then at 25 °C for 10 min, after which acid-insoluble radioactivity was measured. *a*, Effect of the antibody on α-amanitin-sensitive RNA synthesis; *b*, effect of antibody on α-amanitin-resistant RNA synthesis. ●, ▲, Antibody; ○, △, normal IgG.

The question arises as to whether or not S-II is a component of the multimeric proteins of RNA polymerase II. We therefore tested the inhibitory effect of the antibody on purified RNA polymerase II. For this, we preincubated a fixed amount of purified RNA polymerase II with increasing amounts of the antibody at 0 °C for 30 min and then shifted the temperature to 37 °C and measured the incorporation of ^{3}H-UTP into RNA in 60 min. As a positive control, we measured the effect of the antibody on the stimulatory activity of S-II. As shown in Fig. 2, RNA polymerase II itself was not affected appreciably by either the antibody or normal IgG, whereas the stimulatory activity of S-II was specifically inhibited by the antibody but not by normal IgG. These results show that the antibody inhibits the activity of S-II. If S-II were an intrinsic component of multimeric proteins of RNA polymerase II, the antibody should have at least partly inhibited the activity of RNA polymerase II. It is possible that S-II is a component of native RNA polymerase II, but that it was split off during isolation of the enzyme because when multimeric proteins are extensively purified their subunits are sometimes lost during the purification step, as in the case of σ factor of *Escherichia coli* RNA polymerase[15]. However, in this study we used partially purified RNA polymerase II (ref. 16), which was more likely to retain an intact molecular structure than completely purified enzyme. Thus, the fact that the antibody did

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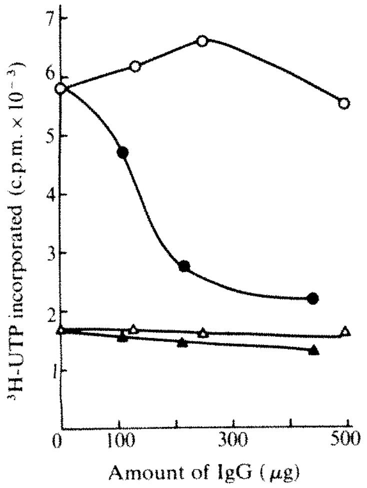

Fig. 2 Effect of the antibody against S-II on partially purified RNA polymerase II. The reaction mixture contained in a total volume of 250 μl, 40 mM Tris-HCl, pH 7.9, 3 mM $MnCl_2$, 4.6 mM $MgCl_2$, 50 mM $(NH_4)_2SO_4$, 0.068 mM EDTA, 4 mM β-mercaptoethanol, 0.25 mM each of ATP, GTP and CTP, 0.025 mM UTP, 0.5 μCi of ^{3}H-UTP (20 Ci $mmol^{-1}$), 5 μg of Ehrlich ascites tumour DNA, 20 units of RNA polymerase II (see ref. 16), 25 units of S-II when present (see ref. 12) and the indicated amount of antibody against S-II or normal IgG. The mixture was preincubated for 30 min at 0 °C, then at 37 °C for 60 min, and then acid-insoluble radioactivity was measured. ●, Antibody + S-II; ○, normal IgG + S-II; ▼, antibody, no S-II; △, normal IgG, no S-II.

not inhibit the partially purified RNA polymerase II strongly suggests that S-II is not an intrinsic component of RNA polymerase II.

From the results obtained here using specific antibody against stimulatory factor S-II, two important conclusions could be drawn about eukaryotic transcription. First, in the process of transcription α-amanitin-sensitive, nuclear RNA synthesis is not a simple process catalysed by RNA polymerase II, but in some way involves a regulatory protein(s) such as S-II. Second, the protein factors reported to stimulate RNA polymerase II *in vitro* are not necessarily components of the multimeric proteins of RNA polymerase II, but may exist as independent proteins that regulate transcription by interacting with the enzyme. Thus, in reconstruction of an accurate transcriptional system *in vitro* using purified RNA polymerase II and deproteinised DNA as template, attention must be paid to the effect of these stimulatory factors. This is the first report of evidence that a stimulatory factor is actually involved in nuclear RNA synthesis.

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Phenotypic suppression and misreading in *Saccharomyces cerevisiae*

A NUMBER of antibiotics and other inhibitors have been useful in genetic and biochemical analyses of the protein-synthesising machinery of prokaryotic and eukaryotic organisms. Aminoglycoside antibiotics have been shown to be particularly helpful in this respect, especially in identifying ribosomal protein cistrons in bacteria. The aminoglycosides cause extensive misreading of the RNA code words *in vitro*[1] and suppress many nonsense and missense mutations in *E. coli*[2] phenotypically. The misreading observed in cell-free translation is believed to be the basis for the phenotypic suppression, although the exact mechanism is not known. Apart from a report of suppression of a single mutation in yeast by streptomycin[3], there have been no demonstrations of phenotypic suppression in eukaryotic organisms. Earlier studies of mistranslation *in vitro* in eukaryotic systems indicated that this phenomenon is rare. Streptomycin did not cause misreading with cytoplasmic ribosomes of rat liver[4], rabbit spleen[5], chicken liver[6] or yeast [7]; neomycin had no effect in rabbit reticulocyte extracts[4] and only a slight effect in the yeast and chicken liver systems[6,7]. These results suggested that translation in higher organisms functions with higher fidelity than that in bacteria. Nevertheless, aminoglycoside antibiotics have recently been shown to cause extensive translational misreading *in vitro* in systems derived from *Tetrahymena*[8], wheat embryo[9], and cultured human cells[10].

We report here that several aminoglycoside antibiotics (including hygromycin B, lividomycin, paromomycin and neomycin) cause phenotypic suppression of UAA (ochre), UAG (amber), and UGA mutations and also promote misreading *in vitro* in the simple eukaryotic organism *Saccharomyces cerevisiae*. Mutants resistant to hygromycin B have been isolated which exhibit altered levels of antibiotic-induced phenotypic suppression.

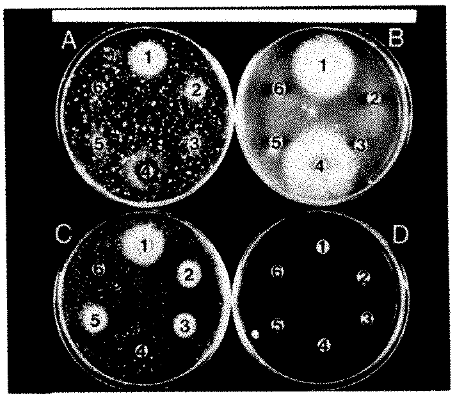

Fig. 1 Test for phenotypic suppression of an UAA (A) and an UAG (B) mutation by various aminoglycoside antibiotics. Strain A carrying an ochre (UAA) mutation, *trp5-48*, and strain B carrying an amber (UAG) mutation, *met8-1*, were grown in yeast extract–peptone–dextrose medium to stationary phase. Cells were collected by centrifugation, washed twice with sterile water and resuspended in water. Approximately 7.5 × 10^6 cells were spread on synthetic agar media. Sterile paper disks were placed as shown on which 500 μg (25 μl of 20 mg ml^{-1} aqueous solution) of the indicated antibiotics were dispensed. The antibiotics used were: 1, paromomycin; 2, lividomycin A; 3, gentamicin C_{1a}; 4, hygromycin B; 5, kanamycin B; and 6, kanamycin C. Petri dishes were incubated at 30 °C for 6 d and then photographed. The white growth surrounding a disk indicates phenotypic suppression. Strains C and D are mutants (see text) resistant to hygromycin B isolated from strains A and B, respectively. Tests with C and D were done exactly as described above for A and B.

Antibiotics were tested for phenotypic suppression of nonsense mutants of yeast as shown in Fig. 1; higher concentrations of antibiotics were also tested. Of 32 nonsense mutations tested, 18 were suppressible (Table 1) by one or more of the drugs. Only a few cases were found where a mutation not suppressed initially was suppressed when higher concentrations of antibiotic were used. The UAA mutation *trp5-48* and the UAG mutation *met8-1* were the most readily suppressible mutations of their respective classes. We therefore used these two mutations to test a variety of aminoglycoside antibiotics for their ability to cause phenotypic suppression (Table 2). Although paromomycin, hygromycin B and lividomycin B were generally the most efficient in causing phenotypic suppression, no clear-cut structure–activity relationship was evident. It has been reported[8-10] that only those aminoglycoside antibiotics that contain a 6′-hydroxyl group on the amino sugar linked to the 4-position of 2-deoxystreptamine were highly active in promoting mistranslation in eukaryotic cell-free systems. If suppression of auxotrophic mutations by the antibiotics in yeast is due to mistranslation, this structure–function relationship does not always apply. For example, in Fig. 1 (A, C) it can be seen that kanamycin B is substantially more active than kanamycin C; the reverse is predicted from *in vitro* mistranslation studies. In addition, several of the gentamicins were active.

Table 1 Nonsense mutations tested for phenotypic suppression

Mutation type	Suppressed*	Not suppressed
UAA	*trp5-48, lys1-1, leu2-1* *met15-30, met15-49,* *met15-85, met15-95,* *met15-119, met15-131*	*his5-2, ade2-1, arg4-17* *can1-100, met15-47,* *met15-70, met15-107*
UAG	*met8-1, ilv1-1, aro7-1* *ade3-26, met15-44* *met15-46, met15-72,* *met15-84*	*trp1-1, met15-21,* *met15-76*
UGA	*leu2-2*	*his4-166, his4-260*

* Suppressible by one or more antibiotics listed in Table 2.

Although most of the nonsense mutations were suppressed by one or more of the antibiotics, phenotypic suppression of missense mutants was rare. No auxotrophic mutations in yeast are known to be missense on the basis of amino acid substitution or nucleotide sequence data. A number of osmotic-remedial and/or temperature-sensitive mutants are, however, believed to be missense. Paromomycin, which is active on all phenotypically suppressible nonsense mutants (Table 1) was tested for suppression of isogenic series of missense mutants in the *met15* locus. Only one (*met15-33*) of 34 could be weakly suppressed. This mutant is osmotic-remedial, shows interallelic complementation and was not suppressible by amber and ochre suppressors[11]. The proportion of missense mutations that are suppressible in bacteria is lower than that of nonsense mutations[12], but it seems to be much higher than in yeast. The frequency of phenotypically suppressible mutants in a random sample of mutants was relatively low; we found only ten such mutants in a collection of 337 independent auxotrophic mutants isolated from a prototrophic strain.

Some of the ochre (UAA) mutants that we examined contain the cytoplasmic determinant ψ, which increases efficiencies of ochre[13,14] and some frameshift[15] suppressors but has no effect on amber suppressors[13]; ochre mutations were more readily suppressed by antibiotics in ψ^+ strains than in ψ^- strains. Since all the amber mutants were ψ^-, and the ψ state of UGA mutants was not determined, we do not know if the ψ^+ genetic determinant facilitates phenotypic suppression of UAG and UGA mutants.

Of the aminoglycosides listed in Table 2, only hygromycin B (ref. 16) and destomycin A (A. S., unpublished) inhibit the growth of *S. cerevisiae* on glucose nutrient medium. These are structurally similar, destomic acid-containing antibiotics. We have selected a large number of mutants resistant to hygromycin B; they are recessive and represent at least three complementation groups. The mutants exhibited a wide variation in the level of phenotypic suppression, ranging from the wild-type response to complete restriction. Two examples are shown in Fig. 1. The resistant mutant (C) isolated from the *trp5-48* strain (A) had a reduced level of suppression by hygromycin B. On the other hand, the hygromycin B-resistant mutant (D) isolated from *met8-1* strain was not detectibly suppressed by any of the antibiotics active on the parent strain (B).

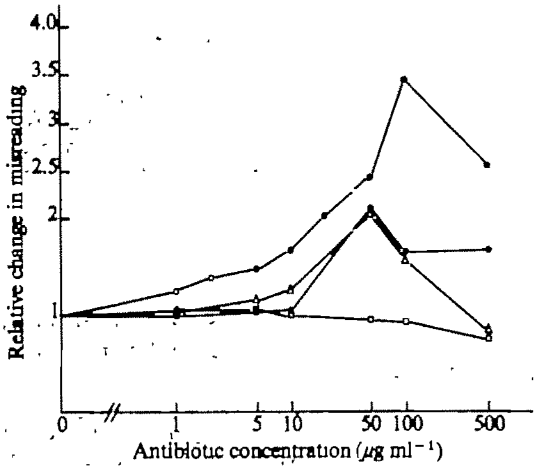

Fig. 2 Effect of aminoglycoside antibiotics on misreading in poly(U)-directed yeast cell-free protein synthesis. The 80S ribosomes and supernatant factors were prepared from wild-type yeast as described earlier[17]. The reaction mixture for poly(U)-directed incorporation of phenylalanine and leucine contained, in 50 μl: 50 mM Tris-hydrochloride, *p*H 7.7, 12.5 mM magnesium acetate, 80 mM KCl, 4 mM creatine phosphate, 40 μg creatine phosphokinase ml^{-1}, 250 μg yeast tRNA ml^{-1}, 1 mM dithiothreitol, 2 mM adenosine 5′-triphosphate, 0.1 mM guanosine 5′-triphosphate, 2 μM ^{14}C-phenylalanine (specific activity 486 mCi mmol^{-1}), 2 μM ^{3}H-leucine (specific activity 8.8 Ci mmol^{-1}), 300 μg poly(U) per ml, 1 mg ribosomes per ml, 2 mg supernatant enzymes per ml. Reactions were carried out at 30 °C for 45 min. 50 μl of 1 M NaOH was added and the mixture was incubated for an additional 20 min. After addition of one ml of 10% trichloroacetic acid the resulting precipitate was collected on glass-fibre filters (Whatman GF/C), washed with 5% trichloroacetic acid and dried. Radioactivity was determined in a toluene-based liquid scintillation solution. Absolute amounts of poly(U)-directed incorporation of phenylalanine and leucine were determined. The data are presented as ratios of misreading observed in the presence of the antibiotics to that found in their absence. As a typical value, the ratio of 1 corresponds to misreading (Leu/Phe ratio) of 4×10^{-3} which in turn corresponds to the incorporation of 50 pmol phenylalanine and 0.2 pmol leucine. ○, Hygromycin; B; ●, lividomycin B; △, paromomycin; □, streptomycin.

We have tested several aminoglycoside antibiotics for translational misreading, as detected by incorporation of leucine directed by poly(U) which does not contain leucine condons. As shown in Fig. 2, hygromycin B, paromomycin and lividomycin B, all of which caused phenotypic suppression, stimulated misreading 2- to 3.5-fold. The same drugs caused misreading when added to a natural mRNA-stimulated system (L. Gritz, unpublished observation). Streptomycin, which does not suppress any of the nonsense mutations, had no effect on eukaryotic translation. The mechanism of misreading by aminoglycoside antibiotics is not known; the interactions between these drugs and pro- and eukaryotic ribosomes are presumably different, since different structure–function relationships are involved. The analysis of the ribosomes of resistant mutants should be

Table 2 Aminoglycoside antibiotics tested for phenotypic suppression activity

Antibiotic class	Active	Not active
The 4,5-disubstituted deoxystreptamine antibiotics	Neomycins B and C, lividomycins A and B, paromomycin	Butirosins A and B
The 4,6-disubstituted deoxystreptamine antibiotics	Kanamycins A and B, tobramycin, gentamicins X_2, C_{1a}, C_2, A_1; sisomicin	Kanamycin C, gentamicins A, B C_1, A_2
The monosubstituted deoxystreptamine antibiotics	Destomycin A, hygromycin B	
Others		Fortimicin A, kasugamycin, streptomycin

profitable in this respect. Hygromycin B has been shown to be an inhibitor of translocation in eukaryotic translation[18] and slowing of this process by the drug may increase the rate of incorporation of incorrect amino acids.

Our studies provide a basis for the genetic and biochemical analysis of mutants which may affect fidelity of translation in yeast and it should be possible to isolate and analyse mutants of the ribosomal ambiguity type in *S. cerevisiae* that have been exploited by Gorini and his coworkers in *E. coli*. In addition, antibiotic-induced phenotypic suppression may provide a convenient and quick screening method for nonsense mutants in eukaryotes. Paromomycin is a potent agent for phenotypic suppression and causes mistranslation *in vitro*, but has no measurable affect on *S. cerevisiae* growth. If the two processes are related, this eukaryote must be capable of tolerating extensive translational errors without apparent harm. This raises an interesting point with respect to the error catastrophe theory of translation errors in ageing.

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Phenotypic suppression of nonsense mutants in yeast by aminoglycoside antibiotics

STREPTOMYCIN, an aminoglycoside antibiotic, can reverse the mutant phenotypes of many nonsense and missense mutations in *Escherichia coli* and in bacteriophage T4. This phenomenon has been called phenotypic suppression, since the mutant phenotype returns after removal of the drug[1]. The most likely explanation for phenotypic suppression is that streptomycin promotes mistranslation *in vivo*, and that acceptable amino acids are inserted into the growing polypeptide chain at the site of the mutant codon. Consistent with this view is the observation that streptomycin causes *E. coli* ribosomes to mistranslate RNA *in vitro*[2,3]. Streptomycin and neomycin have however been found to have no effect in stimulating ribosomes from eukaryotic cells to mistranslate RNA *in vitro*[4,5]. A subclass of the aminoglycoside antibiotics has been shown[6,7] to stimulate eukaryotic ribosomes to misread RNA. The highly active molecules are distinguished in that they contain the drug fragment paromamine (or 3′-deoxyparomamine). We have therefore examined the capacity of various aminoglycosides to suppress mutations phenotypically in the eukaryotic yeast, *Saccharomyces cerevisiae*. The results presented here show that paromomycin, which contains paromamine, is capable of phenotypic suppression of the nonsense mutations in *S. cerevisiae*.

The data of Fig. 1 show the relative ability of various antibiotics to suppress the methionine requirement of a yeast strain carrying the amber mutation *met8-1*. It is clear that in the presence of streptomycin, distilled water or cycloheximide, only rare isolated revertants appear, whereas paromomycin, one of the paromamine-containing drugs, supports a dense mat of growth around a paper disk containing this antibiotic. Although it is not obvious in the figure, neomycin causes a limited ring of growth around the disk.

Singly-cloned cells from the plate containing paromomycin were isolated and tested for their methionine requirement. All of the tested clones were still auxotrophic for methionine, indicating that paromomycin had not induced any permanent revertants. Additional nonsense mutants were tested for their ability to be phenotypically suppressed. The results (Table 1) indicate that 14 of the 17 mutants tested can be phenotypically suppressed with paromomycin, whereas only six mutations are suppressed with neomycin, albeit weakly. The mutations suppressed by paromomycin represent lesions in eight different metabolic pathways and in 11 different structural genes. It is therefore highly unlikely that paromomycin is directly supplying the growth requirements for all of the mutants. Paromomycin

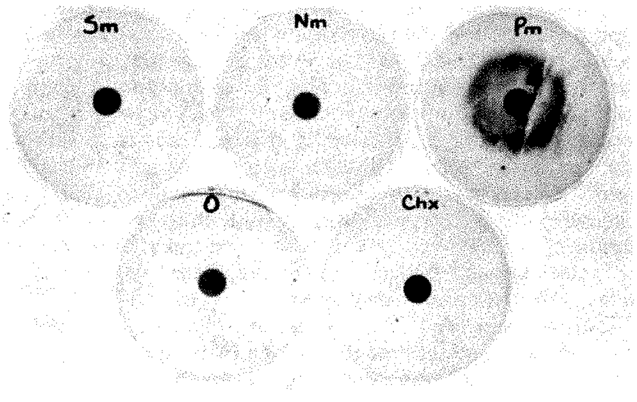

Fig. 1 Approximately 10^7 cells were plated on synthetic media[13] containing no methionine. A paper disk, saturated with 7 μmol (5 mg except cycloheximide 2 mg) of antibiotic, was placed in the centre of each plate and the plates were incubated at 30 °C for 3 d. Sm, Streptomycin; Nm, neomycin; Pm, paromomycin; Chx, cycloheximide; O, distilled H_2O.

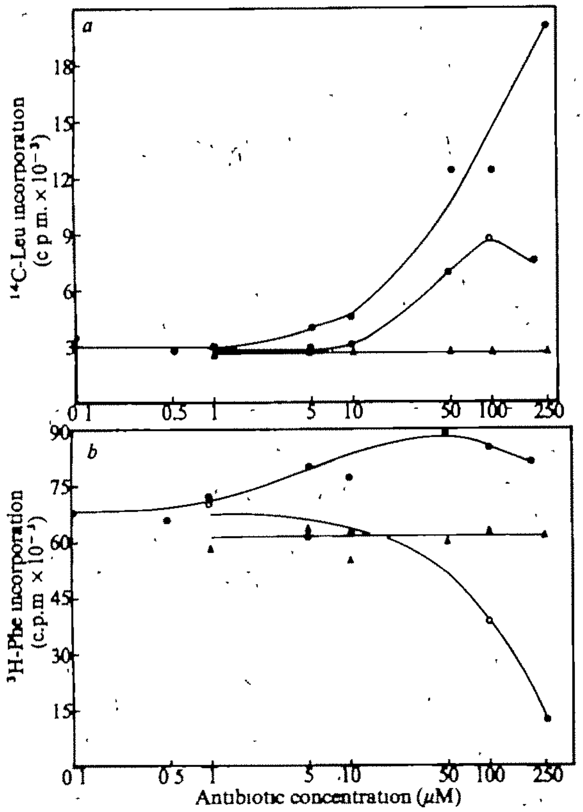

Fig. 2 Yeast cells (strain HL100-1C) were grown in standard nutrient medium (1% yeast extract, 2% peptone, 2% dextrose) to 8×10^7 cells ml^{-1}, collected and washed twice with distilled H_2O. The cells were resuspended in homogenising buffer (20 mM HEPES, 4 mM Mg acetate and 100 mM KCl, pH 7.6) and disrupted with glass beads in a Braun mechanical cell homogeniser. The preparation of the extract and the conditions for cell-free protein synthesis are described elsewhere[6]. The final concentrations of Mg acetate and KCl in these yeast cell-free systems were 15 mM and 100 mM respectively. *a*, Poly(U)-dependent misincorporation of leucine; paromomycin (●), neomycin (○); streptomycin (▲). *b*, Poly(U)-dependent incorporation of phenylalanine, paromomycin (●), neomycin (○), streptomycin (▲).

can also suppress all three types of nonsense codons, UAG, UAA and UGA (Table 1). The strongest growth response to paromomycin was given by the markers *met8-1* and *trp5-48*, neither of which mutation could be suppressed by streptomycin (Fig. 1 and data not shown).

Growth in liquid cultures indicates that paromomycin can exert suppression at concentrations as low as 50 μM (35 μg ml^{-1}). The concentration of paromomycin for optimal suppression of most mutations is approximately 100 μM.

Cell-free translation systems were prepared from yeast and the ability of paromomycin, neomycin and streptomycin to cause *in vitro* misreading was measured by the misincorporation of leucine with a poly(U) template (UUpyr codes for phenylalanine, whereas CUN and UUpur code for leucine). The data in Fig. 2*a* show that paromomycin is active in stimulating leucine errors, yet streptomycin has no activity. Neomycin can stimulate leucine errors, although its activity is substantially weaker than that of paromomycin, especially at low concentrations. The data of Fig. 2*b* show that concentrations of neomycin which stimulate misreading also inhibit protein synthesis, whereas paromomycin stimulates mistranslation without inhibiting cell-free protein synthesis. This inhibition may account for the very limited phenotypic suppression seen with neomycin, even though the drug has some misreading capacity *in vitro*.

Phenotypic suppression can occur by several distinct mechanisms. The phenotypic reversal of mutant characters in yeast by hypertonic media is interpreted as an elevation of the osmotic pressure optima of certain mutationally altered proteins[8]. 5-Fluorouracil and related analogues are believed to reverse mutant phenotypes in bacteria[9,10] and in *Neurospora crassa*[11] temporarily, by incorporation into mRNA, resulting in the pairing of 5-fluorouracil containing codons with incorrect tRNAs.

Table 1 Phenotypic suppression of nonsense mutations in yeast by aminoglycoside antibiotics

Marker and type	Growth supported by antibiotic Paromomycin	Neomycin
UAG		
met8-1	+	trace
aro7-1	+	trace*
ilv1-1	+	0
trp1-1	+	0
ade3-26	+	0
UAA		
lys1-1	+	0
trp5-48	+	trace
ade2-1	0	ND†
his5-2	trace*	0
ura4-1	0	ND
lys2-1	+	0
ilv1-2	+	0
arg4-17	0	ND
leu2-1	+	trace
UGA		
leu2-2	+	trace*
lys2-101	+	trace
his4-166	trace*	0

Nonsense mutations in *S. cerevisiae* which can be suppressed with paromomycin or neomycin. Cells were plated on the appropriate omission media for the indicated markers and incubated for 7 d with antibiotic containing disks as described for Fig. 1.

* Phenotypic suppression of the indicated marker only seen in cells containing the extrachromosomal element ψ^+ (in preparation).

† Not determined.

Since the paromamine-containing aminoglycosides bind to the cytoplasmic ribosomes of *Tetrahymena* and wheat embryos (unpublished observations), and since these drugs presumably bind to the cytoplasmic ribosomes of yeast, the suppression of many nonsense mutants by paromomycin reported here suggests that phenotypic suppression in eukaryotes can occur with agents which affect directly the functioning of the ribosome. It should be noted that Bayliss and Vinopal[12] reported the suppression of an *his1* missense mutant with streptomycin; however, extremely high concentrations of streptomycin were required to exert this suppression. It seems as if phenotypic suppression requires a 300-fold higher concentration of streptomycin, relative to paromomycin.

The paromamine-containing drugs should thus be useful in the isolation and characterisation of ribosomal mutants of eukaryotic cells which are altered in their translational fidelity. In addition, these agents should be helpful in the isolation of nonsense mutants and nonsense suppressors in eukaryotic organisms other than yeast. Most importantly, however, the paromamine-containing aminoglycosides should make possible a close examination of the consequences of protein synthesis errors in eukaryotic organisms.

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X-ray absorption edge fine structure spectroscopy of the active site haem of cytochrome *c*

THE new technique of extended X-ray absorption fine structure spectroscopy (EXAFS)[1] offers promise of detecting small changes in conformation around a central metal atom at the active site of a metalloenzyme, and of relating such changes to the catalytic mechanism. An advantage of EXAFS studies over X-ray diffraction is that they can be carried out in solution at physiological pH where the protein can freely interact with its substrate. We describe here EXAFS measurements aimed at detecting conformational changes during reduction of horse heart cytochrome *c*.

Figure 1*a* shows the EXAFS-spectrum of reduced cytochrome *c* at pH 7, 20 °C, plotted against wave vector $\mathbf{k} = [0.263\,(E - E_0)]^{1/2}$. E and E_0 are the X-ray photon and iron K-edge energies in eV respectively. X-ray photons with energies comparable to or larger than the Fe-1s-electron binding energy (E_0) are absorbed by the iron thereby promoting the 1s electron to empty bound states (K-edge region) or into the continuum (EXAFS region). Two kinds of information can be deduced from an X-ray absorption spectrum. First, from the K-edge region information regarding the electronic state of the iron in the porphyrine frame and second, from the oscillatory region (EXAFS region) the mean bond lengths between the iron and its nearest neighbours (relative to a model compound used as standard). The technique has been reviewed in detail elsewhere[1].

Table 1 $R_1' = R_1 - a/2$ for the first coordination shell [four pyrrole nitrogens, one nitrogen from His 18 and one sulphur from Met 80 (at low pH) or one nitrogen from Lys 79 (at high pH)]

Reduced cytochrome *c*, pH 7*†	1.47 ± 0.03 Å
Oxidised cytochrome *c*, pH 7*	1.47 ± 0.03 Å
Oxidised cytochrome *c*, pH 10	1.40 ± 0.03 Å

Values as obtained from maximum energy transforms[7]. The individuals atoms of the first coordination shell cannot be resolved.

* In 0.1 M sodium phosphate buffer.

† Reduction was achieved by adding an equimolar amount of L ascorbate.

‡ In 0.1 M glycine buffer.

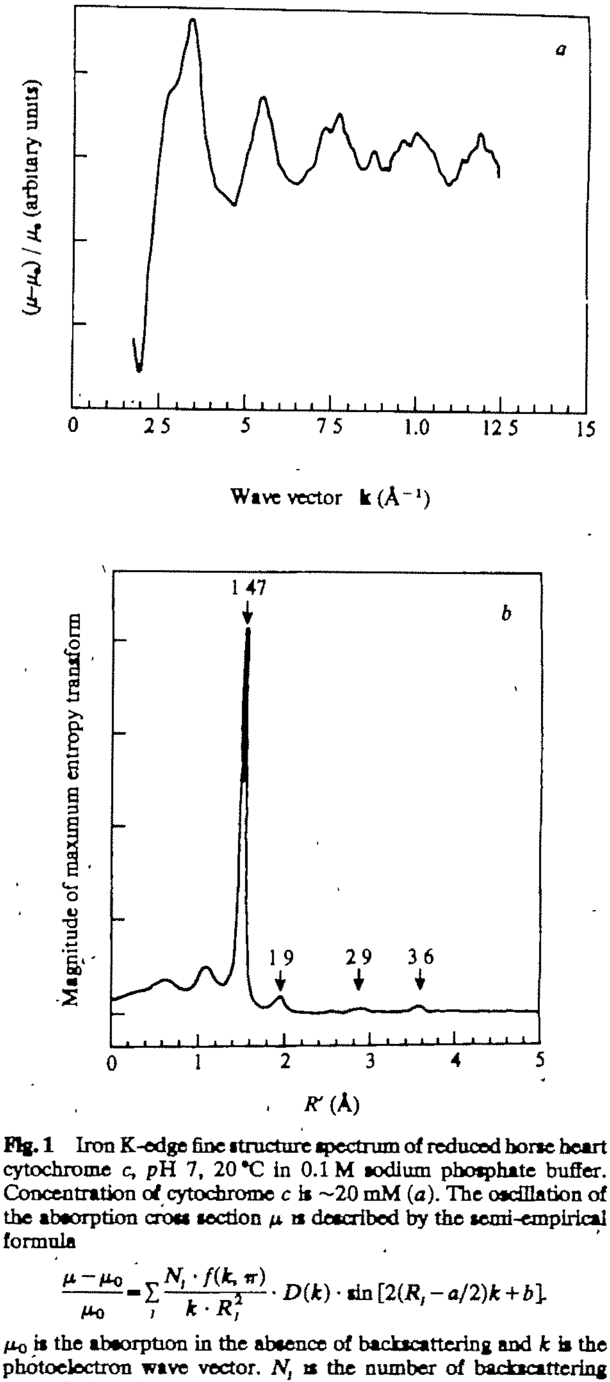

Fig. 1 Iron K-edge fine structure spectrum of reduced horse heart cytochrome *c*, pH 7, 20 °C in 0.1 M sodium phosphate buffer. Concentration of cytochrome *c* is ~20 mM (*a*). The oscillation of the absorption cross section μ is described by the semi-empirical formula

$$\frac{\mu - \mu_0}{\mu_0} = \sum_i \frac{N_i \cdot f(k, \pi)}{k \cdot R_i^2} \cdot D(k) \cdot \sin\,[2(R_i - a/2)k + b].$$

μ_0 is the absorption in the absence of backscattering and k is the photoelectron wave vector. N_i is the number of backscattering atoms in the spherical shell of radius R_i surrounding the absorbing iron. $f(k, \pi)$ and $D(k)$ are the back-scattered amplitude and the Debye–Waller factor respectively. The maximum entropy transform[7] of the spectrum will as a function of the transformation parameter R' show peaks at $R_i' = R_i - a/2$ (*b*). The phase shift $a/2$ is given by the electronic inner shell properties of the absorbing and backscattering atoms and was therefore assumed to be the same for all samples measured. Changes in R_i' are then equal to a corresponding decrease or increase in R_i. The two small peaks in the transform at $R' = 0.6$ Å and 1 Å are spurious and come from an improper background removal (μ_0).

In native conditions (pH 7, 20 °C) the iron of cytochrome *c* is sixfold coordinated. Positions five and six are occupied by the ε-nitrogen of His 18 and the sulphur of Met 80 (ref. 2). In this state it can be reversibly reduced and oxidised. The molecular details of this process are not fully understood. From antibody Fab fragment binding studies[3] it is known that human cytochrome has two different binding sites for the cytochrome *c* oxidase and the succinate cytochrome *c* reductase. The question has been raised as to whether there is a conformational change on reduction[2] which modulates the binding affinity of the two sites to their respective substrates. The latest X-ray diffraction results[4] on cytochrome *c* have a resolution of 2 Å and report the structures of ferric and ferrous cytochrome *c* from tuna which at this resolution do not differ significantly apart from the conformations of a few surface side chains. The early ferric horse heart map[2] showing considerable orientation changes in aromatic side chains and a slight main chain rearrangement to the first ferrous tuna map[5] is now believed to be incorrect.

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The local resolution that can be obtained in EXAFS measurements is considerably higher than by X-ray diffraction. Any conformational change, occurring on reduction is expected to be triggered by the haem. Table I shows the uncorrected values R'_1 (see legend to Fig. 1 for the definition R' of R'_1) for the distance of the first coordination shell from the iron. These values were obtained by calculating the maximum entropy transform[6,7] of the EXAFS spectra as described previously[8]. The magnitude of the transform is plotted as a function of the transformation parameter R' in Fig. 1*b*. We estimate from their reproducibility that the R'_1 values are accurate to ±0.03 Å. Within these limits we cannot see a conformational change in the haem region on reduction. On the other hand, H-exchange experiments[9] on horse heart cytochrome *c* show that the number of slowly exchanging amide protons is significantly higher for the reduced protein. This is interpreted by the authors[9] as unambiguous indication of a conformational rearrangement. In view of the current high resolution EXAFS results we would rather suggest a change in the amplitudes of some low frequency vibrations. The oxido-reduction then shifts the equilibrium between two conformers

$$R^{2+} \leftrightarrow F^{3+}$$

(R standing for rigid and F for flexible) with almost the same time averaged coordinates. Such a 'conformational' transition can be brought about by a variation in bond density (rather than bond length) between the iron and its ligands. It is known that the haem plays an important part in stabilising the folding and that apo-cytochrome *c* does not fold at all[10]. Also the binding of *N*-acetyl-DL-methionine to the haem octapeptide (residues 1–8) has a smaller dissociation constant for reduced octapeptide[11]. Similarly in ferric cytochrome *c* the sulphur atom of Met 80 ligating the haem at neutral *p*H is replaced at high *p*H by a nitrogen (most likely from Lys 79)[12]. This exchange does not occur for ferrous cytochrome *c*. Table 1 gives the value of R' for this high *p*H ferric form of cytochrome *c*. The apparent shortening of the first coordination shell distance by 0.07 Å is marginal in view of the estimated uncertainty of ±0.03 Å for R'. Whether the change in vibrational state or flexibility can alter physical properties such as the specific binding affinity has to await further elucidation.

Fig. 2 Iron K-edges and K-edge derivatives of reduced and oxidised cytochrome *c* and *p*H 7, 20 °C in 0.1 M sodium phosphate. Cytochrome *c* concentration is 20 mM.

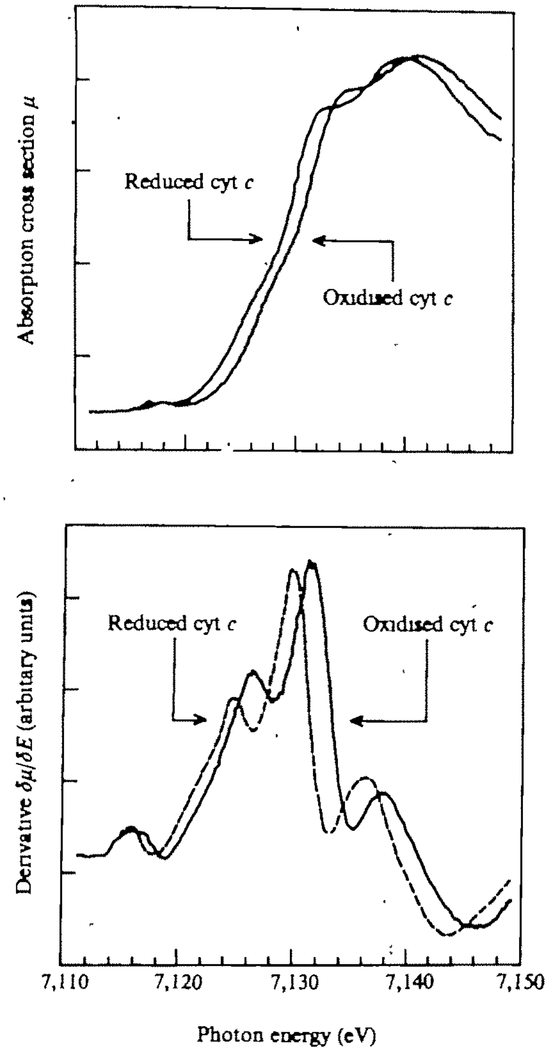

Table 2 Cytochrome *c* K-edge inflection point energies relative to the third inflection point of the K-edge of an iron foil (defined as 7130 eV)

	Oxidised cytochrome *c*		Reduced cytochrome *c*	
	1s–4s	1s–4p	1s–4s	1s–4p
*p*H 1.5	−2.4 eV	+1.5 eV	Unstable	
*p*H 7.0	−3.4 eV	+1.6 eV	−4.8 eV	+0.2 eV
*p*H 10.5	−3.1 eV	+1.7 eV	−4.6 eV	+0.4 eV

Buffers: 0.1 M glycine (low and high *p*H) and 0.1 M sodium phosphate. Cytochrome *c* concentrations range from 20 mM to 35 mM. No effect of the concentration on the K-edge positions could be detected.

Figure 2*a* shows the Fe-K-edge spectra of reduced and oxidised cytochrome *c* at *p*H 7. The edge structure is enhanced by differentiation with respect to photon energy (Fig. 2*b*). Three distinct transitions at photon energies smaller than the 1s electron ionisation energy can be seen. These transitions to empty bound states have been tentatively assigned to the 1s–3d, 1s–4s and 1s–4p transitions[13]. As seen from Fig. 2*b* there is a shift of the 1s–4s and 2s–4p transition by +2 eV on oxidation. This result is highly reproducible and in its most simple interpretation argues against extensive delocalisation of the electron added during reduction. It is in contradiction to results published earlier[13], in which no change in electronic state was detected on oxidation. Moreover, we find practically no effect on the K-edge position when raising the *p*H, resulting in the exchange of the Met 80 sulphur by nitrogen. Table 2 gives the complete set of energies for the 1s–4s and 1s–4p transitions relative to the K-edge energy of an iron foil (7130 eV).

Note that we could not detect any degradation of cytochrome *c* after exposure to the high X-ray flux when checked by the optical absorption spectrum or the kinetics of ascorbate reduction[14].

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matters arising

Was Venus born retrograde?

INGERSOLL AND DOBROVOLSKIS[1] have disputed my theory[2] of the atmosphere of Venus. The key quantity in both analyses is the accelerating atmospheric torque T_A, evaluated in equations (2) and (22) of refs 1 and 2 respectively. The latter expression is determined from observations to within some 30%, while the former contains the unknown 'thermal response time' τ for mass transport in the atmosphere, for which the authors suggest values between some weeks and infinity. But sound waves can travel around the planet within some 10^5 s, hence I expect τ to be about a day; and for this value, both determinations of T_A give the same answer.

The other important quantity is the braking solid body tidal torque T_B which depends on the average value of the quality factor Q divided by the tidal Love number k_2. Estimates for $Q/4k_2$ range from 30 to $\gtrsim 10^5$ (refs 3, 2). In view of the different spin rate, chemistry and thermal history of Venus, a comparison with Earth need certainly not be trusted. An equality $T_A \approx T_B$ can therefore at best be conjectured. In ref. 1, this equality is suggested to hold within less than 2%, an unlikely event without independent justification.

For this and the other reasons put forward in ref. 2, I still believe in the law of isochronism: that all Solar System secondaries were born with a prograde spin period between 5 and 10 h.

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INGERSOLL AND DOBROVOLSKIS REPLY—The questions which have been raised concerning the tidal torque on the atmosphere of Venus[1] deserve some clarification.

The atmospheric torque evaluated in equation (22) of ref. 2 depends on the coefficient of viscosity in the upper atmosphere of Venus. Because this is undoubtedly dominated by eddy momentum transport instead of molecular diffusion, the coupling between the upper and lower atmosphere is uncertain by orders of magnitude, not by 30% as claimed by Kundt.

The thermal response time τ is introduced in ref. 1 to parameterise unmodelled effects which tend to damp the atmospheric tides. It is not necessarily related to mass transport, and has nothing to do with the speed of sound waves (an entirely separate phenomenon). The main conclusions of ref. 1 (see our Fig. 2) are based on the undamped case $\tau \to \infty$.

Regarding the solid body torque, a value for Q of the order of 30 is not only suggested by analogy with the Earth, Moon and Mars, but is required (on the average) if Venus has despun from an originally rapid rotation, whether prograde or retrograde. Nevertheless, it is true that the near-equality (in the opposite sense) of the atmospheric and body tidal torque ($T_A \approx T_B$) is a conjecture. This hypothesis was originally advanced by Gold and Soter[3] to explain the nonsynchronous rotation period of Venus.

These and similar questions are dealt with in greater depth in a forthcoming series of paper, as well as in ref. 4.

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An alternative hypothesis for the origin of West African kimberlites

MARSH[1] suggested in 1973, that the alkaline intrusives along the coast of southwestern Africa occurred where oceanic fracture zones extended under the adjacent continents. Recently Williams and Williams[2] have used this suggestion to attempt to explain the origin of the West African kimberlites. Their interpretation rests heavily on the accuracy of kimberlite and transform fault locations in their Fig. 1, on the assumption of Mesozoic ages for all of their kimberlites, and ignores the important occurrences in the same region of pre-Mesozoic kimberlites and alluvial diamond deposits.

Williams and Williams' hypothesis assumes West African diamond deposits to be Mesozoic, whereas the deposits in Ghana, Upper Volta, the Ivory Coast and Mali are Proterozoic or Infracambrian[3–8]. Their reference to kimberlites in Ghana is incorrect. Their location of a 'kimberlite' in Ghana fits the location of a few diamonds in a stream panning exercise[3] while the largest diamond fields of West Africa (placer), located in southern Ghana, are omitted. Their 'kimberlite' in Nigeria is a lamprophyre dyke located between Zaria and Sokoto (P. J. T. Verheijen, personal communication). Likewise, their postulated inland continuations of transform fault zones contradict without explanation the generally accepted trends[9] of these zones: the Chain and Charcot into the Benue trough,[9] the Romanche into the Keta basin bearing toward Ibadan,[9,10] and the St Paul entering the Ivory Coast east of Sassandra bearing ENE[9,10]. Thus the ages and locations of kimberlites as well as the positions of transform faults and their postulated landward continuations used by Williams and Williams[2] disagree with those established in the literature, with no explanation for this disagreement.

The geographical pattern of kimberlite pipes and the larger diamond placers can be more accurately interpreted (Fig. 1) as occurring along a primary arc running from Ghana through the Ivory Coast and Liberia to Sierra Leone[11]. In such a case the occurrence of young kimberlites is brought into coincidence with all of the much older kimberlites and alluvial deposits in the same general region. In addition there is a possibility of a second, roughly parallel arc extending from northern Ghana through Upper Volta, the Ivory Coast, Mali and Senegal. Known ages of major deposits along the primary arc include the diamonds contained in and above Birimian meta-sediments (2,200 Myr) in Ghana[3,4], the Seguela and Kanangono kimberlites (1,429 Myr) in the Ivory Coast[5,7], and the Sefadu and Koidu kimberlites (140–92 Myr) in Sierra Leone[6]. Along the second arc, the Kenieba kimberlites of Mali have been dated at 1,072 Myr (ref. 5). The geology of these deposits and the age determination techniques and results are discussed elsewhere[3–8]. Such a long history of progressive east to west arcuate diamond-bearing belts requires more than an association between Mesozoic kimberlites and post-Palaeozoic ocean fractures.

An equally viable explanation might be the existence of a long-lived mantle

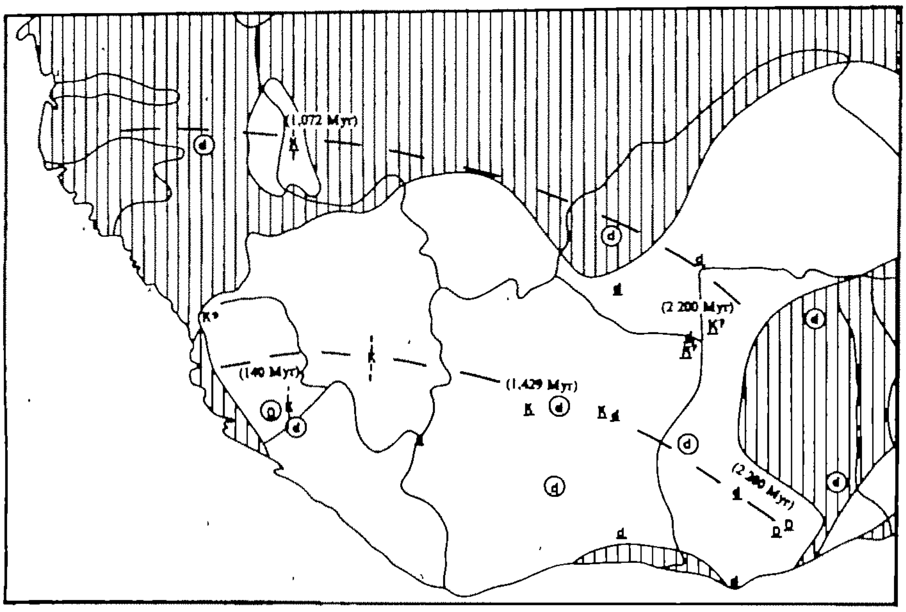

Fig. 1 Diamond and kimberlite occurrences in West Africa. K designates kimberlites (kimberlite belts are denoted with vertical caps and bases), D denotes major secondary diamond deposits, d marks minor secondary diamond occurrences. Underlined symbols are Precambrian in age, other deposits are younger. Circled symbols are alluvial deposits without age determinations; vertically shaded areas are covered with platform or basin sediments. Dates are given for diamond districts in which determinations have been made.

plume[12] producing a kimberlitic magma[13–15]. Then the alluvial deposits and kimberlites of the primary arc could be explained by the slow migration of such a plume from Ghana across the Ivory Coast to Sierra Leone with a long-term relative rate of motion of 0.8 mm yr^{-1}. The deposits on the secondary arc could be explained and would be consistent with a second but possibly weaker plume located north-west from the first plume, roughly in line with the Atlantic continental marginal intersections of the two arcs.

Such a hypothesis is consistent with the longevity, movement, and episodic nature of other hot spots such as the Hawaiian Chain[16]. The intrusion through the continents may be more episodic in nature, being shut off during periods of continental compression and opening up during periods of continental tension. Following the splitting of Africa from South America the rate of relative movement of the plume should have increased greatly. This would place the present position of the hypothetical plume, by extrapolation of the primary arc, at St Paul's Rocks on the Mid-Atlantic Ridge. This is known to be an active hot spot[17] and includes rocks with close affinities to kimberlites and carbonatites[18] as well as uplifted mantle material[19].

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H. R. AND R. A. WILLIAMS REPLY—Hastings and Sharp[1] have attempted to explain the distribution of Precambrian to Mesozoic kimberlites in West Africa by means of two long-lived mantle plumes, the major one tracking from Ivory Coast to Sierra Leone with time. Their criticism of our hypothesis[2] that kimberlites occur along fundamental crustal fractures affected by oceanic lineaments, is unacceptable, especially as our hypothesis has been confirmed by Stracke[3] in south-east Australia, following his visit to Sierra Leone.

Hastings and Sharp have criticised our choice of data, particularly the ages of kimberlites, their location, and that of transform fracture zones. With regard to the first, the reliability of much radiometric dating of kimberlites and other lithologies is fraught with problems[4–7] as shown by kimberlites in Mali, Liberia and Ivory Coast. Argument over the locations of kimberlites and fracture zones probably stems from the small-scale of our Fig. 1. However, some loci of our hypothetical continental continuations of transform fracture zones are ill-defined, but this is due to the scarcity of readily accessible, reliable Geological Survey data in West Africa. We did not include the diamond fields of southern Ghana in our Fig. 1 because we were discussing only the distribution of Mesozoic kimberlites. We are fascinated by the Nigerian kimberlite which has allegedly become a lamprophyre, but this does not subdue our hypothesis, as Scott[8] relates lamprophyres to kimberlites by a simple crystal fractionation process.

With regard to movement of mantle plumes over a period of nearly two billion years, we suspect that an arcuate locus is facile. The movement of the African plate since the Palaeozoic has been roughly northwards, as shown by the building of the Alps, the age succession of the Nigerian Younger Granites, and by palaeomagnetism. If Hastings and Sharp seriously consider that since the mid-Proterozoic plate movement relative to an assumed hot spot may be described as an arc, then we refer them to refs 9 and 10 for illumination.

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The problem of thrown string

BASS AND BRACKEN[1] have proposed a probabilistic model to explain existing experimental results on the average length between the ends of a string when thrown onto a table. The theoretical values obtained from their model are not consistent with the available experimental evidence. However, the model presented seems to be a plausible description of the physical situation. We suggest here a

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modification which takes into account the effect of the desire of the experimenter to obtain a random set of throws. We argue that the experimenter tries in his throws to obtain initial distances between the ends of the string (by initial we mean the distance just before the string begins to fall) which follow a uniform distribution. We contend that in this case the distribution of distances between the ends of the string just before it hits the ground is a mixture of equal proportions of a uniform distribution and the distribution proposed by Bass and Bracken[1]. If this argument is true the density function for λ, the ratio of the distance between the ends of the string at the end of each experiment to its total length, is

$$\omega(\lambda) = \tfrac{3}{4}[\tfrac{5}{3}\arccos\lambda - \lambda(1-\lambda^2)^{\frac{1}{2}}]$$

This gives $E(\lambda) \simeq 0.343$ and $E(\lambda^2) \simeq 0.178$ in striking agreement with the data. Bass and Bracken suggested that a proportion of the throws were conducted close to the table surface and if this were so, their model would give a better agreement with the data. It is easy to show that 38.5% of the throws are required to be near the table surface in order to give a good agreement with the experimental results.

We feel that our assumption is more realistic. However, the controversy on this problem will only be resolved when new experimental results are obtained and all the details of the experiment become available.

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BASS AND BRACKEN REPLY—Synge[1] has noted and rejected in passing the unfounded hypothesis that all possible end-to-end distances D of the string of length L lying on the table are equally probable (leading to $\bar{D}/L = \frac{1}{2}$). A modified version of that hypothesis would be that all possible end-to-end distances of the string in space (before impact with the table) are also equally probable. Taking into account the projections of the string on to the plane table[2], the probability density function for the ratio $\lambda = \bar{D}/L$ is then $\arccos\lambda$ (leading to $\bar{D}/L = \pi/8 \simeq 0.39$). The implausibility of the hypothesis leading to this distribution is apparent from the consideration that the number of configurations of the string consistent with an end-to-end distance x is much greater when x is near 0 then when x is near L.

If a fraction, p, of the throws conformed to our model[2], and $1-p$ to the hypothesis of equiprobability of end-to-end distances (in space), the resulting probability density of λ would be the combination

$$\frac{3p}{2}[\arccos\lambda - \lambda(1-\lambda^2)^{\frac{1}{2}}] + (1-p)\arccos\lambda\,;$$

and if p could be treated as an adjustable parameter, agreement with observations could obviously be improved[3]. We have not been able to find any rational basis for such a combination with $p \neq 1$. In particular, we cannot see that the choice $p = \frac{1}{2}$ would arise from the circumstance that "the experimenter tries in his throws to obtain initial distances between the ends of the string . . . which follow a uniform distribution"[3].

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Is lymphocytic chalone activity restricted to a spermine–protein complex?

THE nature of lymphocytic chalones which are supposed to be tissue-specific but not species-specific inhibitors of cell proliferation, as defined by Bullough[1], remains a matter of controversy. They were characterised as 50–30,000-dalton factors[2,3] but detected also in the lower molecular weight range[4,5]. Recently Allen *et al.*[6] have proposed spermine as one candidate for an *in vitro* chalone activity. Spermine, present in a porcine thymus extracts as a protein complex, inhibited *in vitro* lymphocyte mitogenic response only when fetal calf serum (FCS) was added to the culture medium. FCS seems to act through polyamine oxidation, and its requirement for the inhibitory activity of spermine as well as spermidine was confirmed by Byrd *et al.*[7]. On the other hand, Rijke and Ballieux[8] have found a chalone-like non-dialysable factor in calf thymus extract differing from spermine complex in that its *in vitro* activity was FCS independent.

We have studied a non-dialysable fraction extracted from bovine spleen characterised previously as a lymphocytic chalone[3]. We demonstrated that most of the activity could be dissociated from high molecular weight carriers and recovered in a low molecular weight fraction[5,9] exhibiting chalone activity[10]. After chromatography, this fraction gave a purified spleen extract (PSE) whose effect was then compared with those of spermine and spermidine in mice. PSE (2×25 μg) was injected intraperitoneally. Twenty-four hours later, spleen, testis, kidney and liver cells were incubated with ^{3}H-thymidine. The radioactivity incorporated into DNA of cells was significantly reduced for spleen (39%, $P < 0.005$) but not in liver, testis and kidney cells. PSE treatment depressed both the number of plaque-forming cells of sheep red blood cell-sensitised mice to 80% ($P < 0.001$), as well as cellular immunity to dinitrofluorobenzene up to 51% ($P < 0.01$), as measured by ear thickness in sensitised mice. Conversely, as judged from the same tests, no significant difference was observed between polyamine-treated mice. These results demonstrate that various factors different from spermine exhibit chalone activity.

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ALLEN REPLIES—I believe that inhibitors of lymphocyte mitosis are not restricted to spermine–protein complexes. In our article[1] we described how the activity of lymphocyte chalone preparations of the type originally used by Garcia-Giralt *et al.*[2] and Lenfant *et al.*[3] could be almost entirely attributed to the presence of spermine. We felt that 'these results emphasise the difficulties associated with the isolation of a lymphocyte chalone, but by no means rule out its existence'[1]. Several groups, having heeded our warning and having now eliminated polyamines from their extracts, are finding that inhibitory activity persists. Dr Lenfant's material is interesting in that it seems to be inhibitory both to the proliferation and function of lymphocytes.

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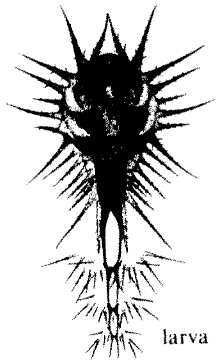

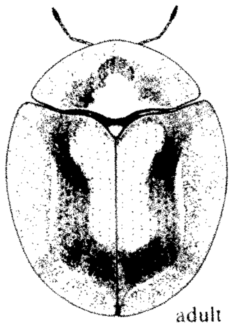

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reviews

Development of preparative techniques

Savile Bradbury

A History of Microtechnique. By B. Bracegirdle. Pp. 359. (Heinemann: London, 1978.) £22.50.

STUDENTS of the history of biological science have devoted considerable attention to the microscope, both to the mechanical development of the stand and to consideration of the resolving power of the optics. Very little by comparison has been written about the equally important topic of the preparation of material for examination. This is an essential part of microscopy, just as much as interpretation of the image produced by the instrument. A knowledge of the development of preparative techniques throughout the period in which the microscope has been in use is, therefore, desirable. Such information, amply provided in this book, helps the historian decide to what extent the performance of the actual microscope was a limiting factor in the acquisition of data, whether there was a conceptual barrier in the mind of the observer or, as may prove likely, it was a combination of these allied to poor and limited preparative procedures. For example, some early techniques for preparing soft tissues such as striated muscle fibres involved their immersion directly in hot balsam resin. As Dr Bracegirdle vividly illustrates, such a preparation dated from 1869 shows little recognisable detail even when viewed by a modern instrument, whereas a microscope of 1826 gives a clear and detailed image of a modern stained preparation of striated muscle. This practical approach characterises the book, whose author examined over 40,000 slide preparations (some 3,500 in detail) during the course of its preparation; in addition he studied microtomes and other instruments in various collections in this country and abroad, and indeed succeeded in operating many of them in accordance with the instructions provided by their inventors.

Although there is clear evidence that microscopes were used to examine biological material from the middle of the seventeenth century onwards, few details of the preparative approaches used at that time survive. This may perhaps be attributed to the fact that very little in the way of specimen preparation was attempted, most observers being content to examine with incident light the surfaces of objects or to study fine dissections at low magnifications. The author considers the early efforts at tissue preparation taking the early period as extending up to 1830. Only sporadic advances such as the invention of clearing agents, the mounting of thin sections of objects between talc covers in wood sliders and methods of micro-injection are to be found during this period. Obviously very few workers were taking microtechnique seriously and although the microtome, one of our most useful preparative tools in microtechnique, had been invented, only three examples (those of Custance, Hill and Adams) are recorded. This early period was, as the author comments, devoted to "specific advances of varying worth, not assimilated into an armoury of techniques generally in use"; some advances of great significance were however made, such as the introduction of the thin glass coverslip in 1789.

The century following the development of the achromatic microscope in 1826 was a time of great experimentation in microtechnique. Many and various reagents were tried, together with a host of embedding media. Some of these proved suitable—for example, paraffin wax and celloidin—and have survived into current use, whereas others—such as shellac, pulped paper, copal varnish and even tinfoil—have proved totally unusable and have disappeared from current practice.

Dr Bracegirdle devotes a long chapter to a discussion of the development of instrumentation in the nineteenth century. This chapter is largely an account of the microtome in its many and various phases and represents fascinating reading for a modern histologist.

The development of suitable chemicals and instruments for specimen preparation naturally led to the production of a large literature, much of it in German, during the nineteenth century. In his survey of this aspect of the subject, the author makes a very interesting comparison between the attitudes in England and on the continent of Europe between 1830 and 1910. In this country it was clear that the gifted amateur reigned supreme in the use of the microscope, pursuing the ultimate in resolution. The perfection of mounts (usually of diatoms) which allowed this seemed to be the aim. On the continent, especially in Germany, the value of the microscope was appreciated for the help that it could provide in elucidating the structure of both normal and diseased tissues. This was reflected in the rapid development of microscopic histology in the medical schools on the continent and by the contributions of workers such as Purkinje, Henle, Kölliker and Virchow.

This is a fascinating book, not only for the historian of science but also for the general histologist. It uses the history of microtechnique, as it developed in the nineteenth century, to pose the question whether many of the workers were not in danger of developing sheer technique and procedure at the expense of careful observation and experiment. Such a question is still relevant today.

The book is well produced and contains both name and subject indices, together with citations of over 1,000 original books and articles. The illustrations are all by the author; many are of material from his own extensive collection and are of a high standard, although some may find the placing of all the plates together in two adjacent signatures irritating.

Here we have a work which is sure to remain the standard reference in this field for many years to come. ☐

Savile Bradbury is Lecturer in Human Anatomy at the University of Oxford, UK.

Implications of artificial intelligence

Computer Revolution in Philosophy: Philosophy, Science and Models of Mind. By A. Sloman. Pp. 304. (Harvester: Brighton, UK, 1978.) Hardback £12.50; paperback £5.50.

THIS is an untidy, un-pompous, exciting, iconoclastic and exasperating book. The author, a professional philosopher as well as a research worker in artificial intelligence, is scathing about much of the prevailing methodologies of science, especially psychology and the social sciences generally, but his approach is perhaps best indicated by the following quotation in regard to philosophy:

> "I am prepared to go so far as to say that within a few years, if there remain any philosophers who are not familiar with some of the main developments in artificial intelligence, it will be fair to accuse them of professional incompetence, and that to teach courses in philosophy of mind, epistemology, aesthetics, metaphysics, and other main areas of philosophy, without discussing the relevant aspects of artificial intelligence will be as irresponsible as giving a degree course in physics which includes no quantum theory."

I am not competent to pass judgment on this sweeping prophecy, but there is no doubt that much interesting and convincing evidence and arguments tending to support it are presented. And on the way, a host of intriguing ideas and speculations, the majority of them not followed up alas, are thrown up.

Here, only a few examples can be given. Few philosophers of mind, not to mention neuropsychologists and experimental psychologists, have yet taken on board the insight provided by the fact that the same cognitive computer programme can be run successfully on different computers with quite different basic components and architecture. In other words, the specifically mental part of the system is in an important sense quite independent of the physical part (which of course is not to say that it can operate without a physical part). How this comes about, the notions of "virtual machine", and of levels of representation of the activity, connected by translators, compilers and interpreters; are of significance here. Consider also the implications for the doctrine of reductionism in the philosophy of science. If biological processes are computational processes running on a physicochemical system, then it is not necessarily the case that the nature of the physical world determines them.

Another important notion for the psychology and philosophy of mind is that of systems and mechanisms which have the property of "mutual recursion", that is, the relationships between any parts of the system are not confined to being disjoint, or part-whole or overlapping, but A can be a part of B which is at the same time a part of A, infinite regresses being avoided by stopping conditions. A characteristically fascinating aside of Sloman's here is the suggestion that neglect of this aspect of the mind might have profound implications for educational practice, in that cumulative educational programmes may be misguided: teachers should not be surprised at pupils getting things wrong when they are in the midst of learning a collection of mutually recursive concepts.

An example of how tantalising the author can be is in a chapter which contains a most original and searching analysis of the relationships between inferences of the analogical type, such as those using diagrams, in contrast to the more usual "logical" types. In the middle he makes the very interesting suggestion, without following it up at all, that the essence of our notion of causality may be the following: we talk of causes when we believe there is a representation of the process (in the analogical or other form) which enables the effect to be *inferred* in the broader sense of "logic" he has been developing.

In the hope that the above has given some sense of the flavour of the book, I shall now give a very brief summary of its contents. The first section deals with the aims of science and its relation to philosophy, putting the emphasis on the study of possibilities rather than the search for laws, and on conceptual analysis; it incidentally argues for a closer community of aims among philosophy, science *and* technology. The next section first attempts (in a very ambitious way) to sketch the structure of a mechanism of general intelligence. Three successive chapters then discuss intuition and analogical reasoning, learning about numbers and its bearing on the many and very diverse philosophical views on the nature of number, and perception as a computational process. It seems clear that the latter topic (in which Sloman's own research has been mainly concentrated) has been the one that has stimulated his thinking most. The final chapter discusses a large set of classical problems of philosophy in the light of our present understanding of artificial intelligence. These include universals, free will and determinism, mathematical knowledge, and aesthetic experience.

The main criticism one might make of the book, as has probably already occurred to the reader of this review, is that it tries to cover too much, with the result that it spreads itself too thin over the large majority of the issues it raises, and gives the impression of making sweeping judgments without sufficient backing. Sloman also has a maddening habit of producing frequently lists (sometimes as long as fifty items) of questions for the reader, most of which are indeed interesting and pertinent ones, but of few of them does he say anything more. In addition, nearly every one of them would probably require at least a day's solid thinking on the reader's part to get any benefit from. Also, future editions of the book (which I am confident will be called for) would be improved by correction of the inordinate number of misspellings, removal of howlers like attributing the explanation of the equivalence of mass and energy to the theory of *general* relativity, or stating that superconductivity was explained before it was observed, and also improving the legibility of the legends of the diagrams.

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Mathematical approach to vectors

Vectors in Three-Dimensional Space. By J. S. R. Chisholm. Pp.293. (Cambridge University Press: Cambridge and London, 1978.) Hardback £15; paperback £4.95.

FEW can appreciate a Beethoven sonata by merely reading the score. It needs to be played by a gifted interpreter. Ease and familiarity with mathematical ideas come quickly to only a few. Many of us need to develop mathematical skills, but such skills are precariously based if we have no appreciation of the underlying mathematical logic. The interpreter is needed here too and J. S. R. Chisholm has demonstrated yet again that he has this gift. His book with Morris *Mathematical Methods in Physics*—now sadly out of print—was a model of its kind: clear and readable but paying attention always to the rigorous basis of the techniques. This new volume is aimed more directly at the mathematics undergraduate but it should be one of the 'lecturer's textbooks' for anyone teaching vectors to scientists and engineers.

Too often a mathematician's approach to vectors begins with an assertion of the general algebra of vector spaces, leaving the student to find analogies, if he can, with the world of three dimensional vector quantities in which he lives. This book maintains a healthy link with intuition. The axioms of vector space algebra are introduced from the start, but the three-dimensional geometrical interpretation of them takes up much of the text, with line-drawings to illustrate many of the arguments.

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David J. Miller

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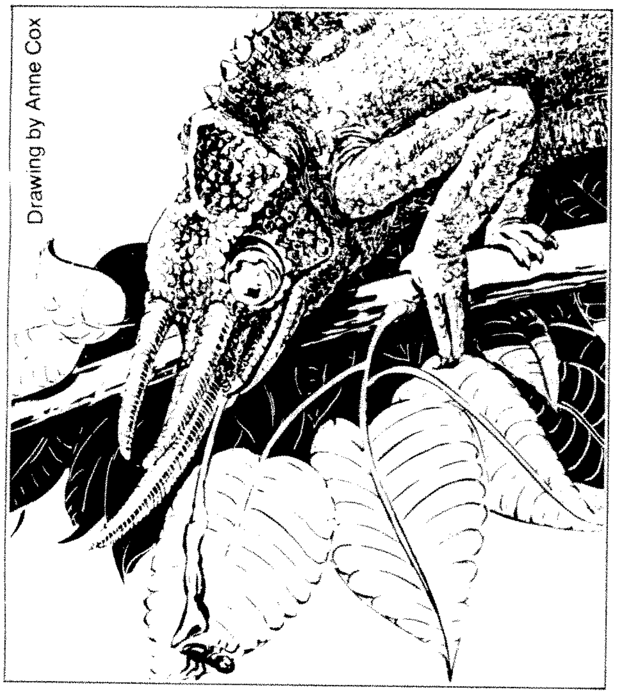

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Biology of cancer

Cancer: A Problem of Developmental Biology. By G. B. Pierce, R. Shikes and L. M. Fink. Pp. 242. (Prentice-Hall: Englewood Cliffs, New Jersey, 1978.) £11.65.

READERS of that neglected masterpiece, *The Crock of Gold* (J. Stephens, Macmillan, 1928), will remember the philosopher's comment (to his wife) on the porridge — "Perfection is finality, finality is death. Nothing is perfect. There are lumps in it." Like the porridge, *Cancer: A Problem of Developmental Biology* has some indigestible lumps in it, but for all that it is a useful book for those for whom it is intended (graduate students). Don't be put off by the modest aims outlined (one hopes by the publisher) on the book cover:

> "The unifying theme of this modern approach to the study of tumors is development and neoplasia, treating carcinogenesis as a model of differentiation rather than as a neoplasmic entity alone. Coverage includes chapters devoted to the early development of neoplasms, tumors as caricatures of tissue renewal, structure and biochemistry of neoplasms, origin of neoplastic stem cells, carcinogenesis, genetics and neoplasia, controls, immunity and neoplasms, metastasis, and caner therapy.
>
> "An appendix concerning various types of tumours also is included. Among the features: Emphasizes the importance of cellular interaction in tumors, showing their cellular heterogeneity and control mechanisms; Presents an excellent introduction to the clinical phenomenology of cancer, placing more specialized aspects in a useful perspective; Organized into a logical, unified, and complete volume that includes the results of most recent research; Provides a complete bibliography of sources of more specialized information; Includes a chapter on therapy and some brief descriptions on the commonly studied tumors."

It does discuss many of these aspects but not in exhaustive detail, in its 199 pages of text. It does not compare in depth with Foulds' *Neoplastic Development* (Academic, 1969) which is still the best general introduction for the specialist to the biology of cancer. *Cancer: A Problem of Developmental Biology* is designed as an introduction to the field and in this I think that it achieves its purpose. For the student of biology with a general interest in cancer or a neophyte about to plunge into some highly specialised branch of cancer research, Pierce *et al.* will provide a useful reminder that there is more to cancer than molecules and also remind its readers that many of the findings of earlier workers are still relevant.

The faults of the book are superficial irritants rather than major defects, but nevertheless they are important, particularly in a book intended for students. The emphasis on the interests of the authors is understandable and not excessive, but the style sometimes grates. There has to be a better way of saying "respond negatively in terms of growth rate", particularly if one wants students to learn conciseness of expression.

A more serious criticism is concerned with minor but important inaccuracies and omissions. It is easy for a reviewer to carp over trifles, particularly when the authors are trying to compress a mass of information into a small space, but it is important not to mislead. A few examples will illustrate the point. The given definition of malignant tumours states that the malignant cells "are intrinsically dangerous because they are rapidly growing and quickly cause cachexia or they invade and metastasize. The intrinsically dangerous cells of malignant tumours invariably destroy their host unless they are either extirpated or killed". Although this is often so, the variation in growth rate of tumours is well known as is the whole concept of latency in tumours and of carcinoma *in situ*, which are in fact discussed elsewhere. The appropriate qualifying clauses would clarify the situation—important for those using the index to look for a definition of malignancy.

On the experimental side the authors suggest that transformation *in vitro* should produce a spectrum of cell lines, some tumour producing, some not—"an idea that has escaped attention", although they quote elsewhere a paper describing this very phenomenon. Sadly (as the paper is one of mine) it is given as a reference to work it does not describe.

In the general discussion on spontaneous transformation *in vitro* the authors fail to mention that this is predominantly a feature of some rodent cells and does not occur in species in which cells have been shown to have a finite lifespan as in Hayflick's experiments with human cells. In the section on genetics and neoplasia a statement is made that "recently, viral particles, similar to those of mouse mammary tumour virus, have been discovered in the milk of a high percentage of Parsi women" (Moore *et al.*, 1971), with the implication that these particles are true viruses and associated with a high incidence of breast cancer in these women. Many would no longer accept these findings. Other examples can be cited.

In spite of the criticisms this could be a useful book but it would be greatly improved by a more critical assessment of statements, definitions and references.

L. M. Franks

L. M. Franks is in the Department of Clarellu Pathology at the Imperial Cancer Research Fund Laboratories, London, UK.

High performance liquid chromatography

High Performance Liquid Chromatography. Edited by J. H. Knox. Pp. 203. (Edinburgh University Press: Edinburgh, 1978.) £5.

THIS book is based on the material of a course on high performance liquid chromatography (HPLC) given in 1977 at the Wolfson Liquid Chromatography Unit, of which Professor Knox is the director. It represents a commendable attempt to put both the theory and practice of the subject on a firm physicochemical foundation, which the student can use to attack his own problems. The main theory of liquid chromatography is compressed into the relatively short chapter 2, followed by successive chapters on the familiar subdivision of chromatographic methods according to retention mechanism, adsorption, liquid-liquid partition, and ion-exchange (including ion-pair) and exclusion chromatography. These topics are very fully treated in the space available, and each chapter includes a table of the appropriate column packing materials commercially available.

Unfortunately the theoretical treatment of chapter 2 is rendered unnecessarily confusing by an unfamiliar notation in which V_m, the volume of eluent within the column, is taken to include both the liquid in the interstitial spaces between the packing particles and the liquid within the stationary phase pores. Although the pore volume cancels out in the definition of phase capacity ratio k' (equation 2.2, p5), it can only be valid as a mean value in the definition of linear flow velocity μ (equation 2.3). It leads to a new definition of zone capacity ratio k'' on p7, which is almost immediately abandoned on p8, leading to the more usual relationships of equations (2.12–2.17). The concept is again introduced in the treatment of the column resistance factor on pp9–10. This notation is all the more surprising, as the authors found it unnecessary in their excellent theoretical treatment in an earlier book.

Perhaps the most unfortunate result appears however in chapter 7 on exclusion chromatography, where an essentially simple concept is confused by the introduction of three definitions for the distribution ratio between the phases (p69). This chapter also illustrates the preoccupation of practitioners of HPLC with the exclusion chromatography of synthetic polymers, usually uncharged and soluble only in organic solvents. Although there is a brief reference to the exclusion chromatography of biopolymers in chapter 7, the treatment is in some respects misleading. For example, in the case of the gel media used with biopolymers, there *is* an increased zone dispersion, which *has* a good theoretical foundation in the polydispersity of retention sites (that is pores) in the stationary phase. This problem has been extensively discussed by Ackers and others. The consequent poor resolution has lead to the virtual abandonment of exclusion chromatography for the determination of the molecular weights of biopolymers by biochemists, who now favour electrophoresis in a continuous molecular sieving medium for this purpose. Also contrary to the impression given on p76, adsorption problems are still severe with many proteins on inorganic exclusion media. Polymer chemists seem to be indeed fortunate in the exclusion media available to them.

Chapters 8–10 give a very good account of the hardware of HPLC, columns, injection systems, pumps and detectors. Perhaps uniquely, this section also includes a discussion on the use of post-column reactor systems, which may be increasingly used in the future to increase both sensitivity and selectivity. Although the authors seem to be optimistic about the future development of many of the presently less common detectors, I feel that the current escalation in the cost of HPLC equipment may put many of these more sophisticated detectors out of the reach of any but specialised laboratories. From this aspect I consider the comparison of analytical methods in Table 11.1 rather biased in favour of HPLC, especially as regards specificity and cost. For example, a good radioimmunoassay can be at least as specific as an HPLC estimation, whereas instrumental costs have been overtaken by inflation during the time taken for publication of the book.

Applications of HPLC, particularly to pharmaceutical and clinical analyses, and a description of the Wolfson departmental course experiments conclude the book. The all-important topic of column packing is excellently covered, although late in the book.

As a comprehensive, modern and methodical treatment of all aspects of HPLC, this book has few rivals, especially at its very modest price.

C. J. O. R. Morris

C. J. O. R. Morris is Emeritus Professor of Experimental Biochemistry in the University of London, UK.

Molecular damage after radiation

Radiation Effects: ESR and ENDOR Analysis. By H. C. Box. Pp. 280. (Academic: New York and London, 1978.) $24.50.

THIS book describes the molecular damage which occurs in carboxylic acids, amides, amino acids, peptides, proteins, pyrimidines, purines and nucleic acids, when these substances are bombarded with high energy radiation. In addition to the immediate effect of the radiation, the secondary processes are described and some overall mechanisms are followed through. Most of this information is derived from a knowledge of what radicals are formed, in what yield, how quickly they react, and with what. It is the unique contribution of electron spin resonance (ESR) supplemented by ENDOR (a special form of nuclear magnetic resonance which uses the ESR signal as a means of detection), which makes this study possible.

The first five chapters describe the characteristics of the ESR and ENDOR methods. In this part of the book, hyperfine structure and g values are treated with fair rigour and the Hückel and INDO methods of computing spin densities are compared with each other and with experiment. The next five chapters are concerned with the radiation effects and mechanisms while the last chapter sets all this into perspective with current and projected work on: radiation modifiers which might be used in cancer treatment; conversion of solar energy into chemical energy; the relationship between radiation-induced carcinogenesis and DNA repair processes; and radiation damage in electron microscopy.

In general the book is well balanced between theory and experiment. It is well written and makes a fascinating account of our knowledge of the molecular events which follow irradiation of biological material. There are lots of well labelled chemical formulae and plenty of tables of data for reference purposes in the text and in appendices; these will make the book valuable for active workers in this field. The reviewer personally favours a slightly more experimental approach and would have preferred to see a few more actual spectra, particularly ENDOR spectra and more details of the assignments on these. Space could have been found at the expense of some of the mathematical detail in the early chapters which many readers more familiar with cell biochemistry might find difficult and unnecessary to follow.

J. F. Gibson

J. F Gibson is Lecturer in Chemistry at Imperial College, University of London, UK.

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obituary

Ivor Isaac

IVOR ISAAC, Professor of Botany in the University College of Swansea and Vice-Principal of the College from 1973 to 1977 died on 4 August 1978 at the age of 63.

He was a Swansea man born, bred and educated in the City. From Dynevor School he entered the University College of Swansea in 1934 and graduated with first class honours in botany. Isaac then trained as a teacher and taught for a short time afterwards but in 1940 he was awarded an A.R.C. Scholarship to work at Cambridge on plant pathology under the supervision of Professor F. T. Brooks. Two years later, Isaac moved to East Malling Research Station as a plant pathologist where he continued his studies for a Cambridge PhD on the plant wilt diseases caused by the fungus *Verticillium:* these studies determined Isaac's subsequent career.

After three years at East Malling, Isaac returned to school teaching at Cheltenham but in 1947 he was appointed Senior Lecturer in Biology at Acton Technical College which was later developed into Brunel University. While there, Isaac was responsible for the planning of the new Department of Biology at Brunel However, in 1950 the opportunity came to return to Swansea first as lecturer in botany and subsequently as reader and professor in the University College. There Isaac played his full part in the development of a rapidly growing department and he pursued his researches on *Verticillium* wilt diseases so successfully that he soon became recognised internationally as one of the leading experts on this important pathogen.

Isaac's early work centred on clarification of the taxonomy of *Verticillium* isolates. At that time, there was considerable confusion because it was not clear whether the two most destructive pathogens in the genus viz. *V. albo-atrum* and *V. dahliae*, were forms of the same organism or were two distinct species. By using morphological, physiological and pathological criteria, Isaac established convincingly that these organisms are distinct species. A distinguished fellow worker wrote subsequently that this work brought order out of chaos.

Isaac continued, with the help of research students, to study many different aspects of the vascular wilt diseases caused by *Verticillium* both in the United Kingdom and overseas. The studies were extended to a wide range of host plants often of great economic importance, such as lucerne and potato. The studies resulted in a continuing series of papers covering the ecology and survival of the fungus in the soil, the etiology of the disease, methods of control and the prevention of dissemination.

Isaac's reputation as a plant pathologist led to invitations to visit and advise in several countries including Italy, Malaya, India and the United States. Shortly before his death he was collaborating closely with the U.S. Department of Agriculture on an outbreak of *Verticillium* wilt on American lucerne crops. He served on several committees concerned with plant pathology and he played a leading part in the establishment of the first International Conference on *Verticillium*.

Isaac was no narrow academic. In his early years he was a very considerable soccer player and he captained both the Cambridge University team and the war-time combined Oxford and Cambridge XI. In later years in Swansea he was always deeply concerned with adult education and he frequently taught extra-mural courses himself. He was a man with many friends. His warm sympathetic personality was combined with high academic ability and clarity of thought; these qualities were much appreciated by colleagues and by students at all levels. He is survived by his wife Catherine, by three sons and a daughter.

J. M. Milton

D. W. Kent-Jones

DR DOUGLAS WILLIAM KENT-JONES, O.B.E., F.R.I.C., died peacefully in Ealing on 31 August 1978, aged 87. He was one of the pioneers of the application of science to cereal technology and was recognised internationally as an authority on cereal chemistry. Indeed his book *Modern Cereal Chemistry* testifies to his immense knowledge of his subject.

In the 1914–18 war he served in the Royal Fusiliers, the Special Brigade of the Royal Engineers and the Royal Flying Corps. It was during this time he claimed he learned his great capacity for getting on with others, and it was this capacity that spurred him throughout his long and active life to give generously of his time to many professional bodies. Thus he was honorary Treasurer of the Royal Institute of Chemistry (1946–53) and its President from 1955–57. He was honorary secretary of the food division of the Chemistry Section of the International Union of Pure and Applied Chemistry, Chairman of the Council of the British Industrial Biological Research Association (1966–70) and its President from 1970–72. In 1971 he was awarded the biannual Gold Medal of the Society of Chemical Industry and in 1974 he received the O.B.E. for his services to the food industry.

During the greater part of this time he was (until he reached the age of 75) the senior partner of the highly successful analytical and consulting chemists, D. W. Kent-Jones and A. J. Amos. After the 1914–18 war he had been chemist, and later director, of a firm in Dover associated with the milling and baking industries and in 1931 he formed the practice, specialising in cereals, first from laboratories in Dover, later in Ealing, and finally the spacious and well-equipped premises in Dudden Hill Lane, London

He learned his baking by working in small bakeries after his days work, and in 1935 published *The Practice and Science of Breadmaking*. From 1926–45 he was the Chief Examiner of Breadmaking to the City and Guilds of London Institute. During 1930–39 he travelled and lectured extensively, becoming involved in Procea Products Ltd. when in the Antipodes and when the Company was formed in the UK he became a director, and finally Chairman, retiring in 1965. In 1938 he was awarded the Belgian Affront medal, and was made Professor *honoris causa* of the Institute des Industries de Fermentation In 1947, at the request of the Bread Manufacturers of New South Wales, he advised on the setting up of the Bread Research Institute of Australia.

Wherever he went he was interested in measuring the qualities of flour and their relation to bread, cake and biscuit baking characteristics and he published many papers on this subject. In 1949 he gave the Cantor Lectures of the Royal Society of Arts. He is well known for the development, with his colleague W. Martin, of the Flour Colour Grader, and the instrumental techniques now used in determining flour quality owe much to him, and his partner's enthusiasm for technological innovations.

Kay-Jay (as he was mainly known) and his wife had no children, but within the family he gave great affection and loyalty; so too did he to his many professional and close friends. He found great joy in the companionship of the Savage Club and after retirement, through being given honorary memberships of the Flour Milling and Baking Industries Research Association, the British Industrial Biological Research Association, the American Association of Cereal Chemists and the Bread Research Institute of Australia, as well as the honorary Fellowship of the Institute of Food Science and Technology.

J. B. M. Coppock

D. M. Dring

Dr D. M. Dring, Mycologist, Plant Pathologist and Quarantine Officer at the Royal Botanic Gardens, Kew, died suddenly after a brain haemorrhage on 26 July 1978 at the age of 46.

Born in Peterborough he went to Exeter University and obtained an external London B.Sc. (1953). From 1953 to 1956 he was an Andrew Simons Scholar at the same University where he worked on *Mycosphaerella* disease of cauliflower for which he was awarded a Ph.D. (1958).

This led to a wider interest in plant pathology and from 1956 to 1960 he was plant pathologist to the government of Ghana with an additional duty to act as Director of the Botanic Garden at Aburi. From 1960 to 1961 he was Agricultural Officer to the United Nations Food and Agricultural Organisation in West Africa working on Kaincope disease, spending much time in the Camerouns and Togo. During his travels he became interested in the larger tropical fungi, especially those bizarre and brilliantly coloured Gasteromycetes belonging to the Phallales.

On return to the United Kingdom he was awarded a Senior Research Fellowship to work for three years at the Royal Botanic Gardens, Kew, on the fungi he had collected in West Africa. Later in 1965, he joined the Scientific Civil Service as a Senior Scientific Officer and became a permanent member of the staff at Kew, where his unique knowledge of plant pathology combined with an interest in taxonomy of higher fungi were invaluable. Indeed his experience of tropical plant pathology ideally fitted him for the job of Officer-in-Charge of quarantine in a rapidly expanding unit, and in 1973 he was seconded briefly to the Government of Fiji to advise on the setting up of a quarantine station.

Donald Dring will be remembered for his publications on Gasteromycete taxonomy which established him as one of the world authorities on this difficult group of fungi. These contributions included floristic studies of the species occurring in tropical Africa and Israel. He also published, with Dr H. Kreisel, a monograph of the genus *Morganella*. However, his chief interest lay in the elucidation of the complex structure, inter-relationships and taxonomy of the phalloids, especially of the family Clathraceae. His magnum opus *Towards a natural re-arrangement of the family Clathraceae* was in an advanced stage of preparation at the time of his death and it is hoped to publish this in due course.

Another of his many scientific activities involved giving a stimulating annual course of lectures on mycology to the student gardeners at Kew. He also took a keen interest in the affairs of the British Mycological Society and served as a co-editor of their Transactions from 1978–1971, and for part of that time as senior editor. His editorial expertise also led to his serving on the editorial board of the *Kew Bulletin*. He also served on the Council of the Systematics Association.

Donald Dring was a congenial colleague who was always happy to take part in and contribute to social occasions. He had a great interest in antiques and enjoyed restoration of period furniture. He leaves a widow, Vivienne, and two daughters.

Derek A. Reid

A. S. Jack

Anthony Jack, member of staff of the MRC Laboratory of Molecular Biology, Cambridge, died of a heart attack on 14 July 1978 at the age of 30.

Born in Newcastle, he was educated at Leeds Grammar School and at Peterhouse, Cambridge, which he entered as an Exhibitioner in 1966. After getting a First in Part II of the Natural Sciences Tripos (Crystallography), he went on to the MRC Laboratory of Molecular Biology to work for a PhD which he received in 1972. He spent two years on a Jane Coffin Childs Research Fellowship at the Gibbs Laboratory, Harvard University, and returned to the MRC Laboratory in 1974 as a junior member of staff.

Tony Jack's research work centred on the structures of large biological molecules and methods of solving them by X-ray analysis. Although a skilful experimenter when the occasion demanded, he was, by temperament and aptitude, more of the analytical type of macromolecular crystallographer, more intrigued by the way to the solution than by the results of the solution itself. Thus it was no coincidence that he was attracted to problems which were non-orthodox at the time. His PhD thesis, following work initiated by Blow, Rossmann and Crowther, deals with the problem of how to use for phase determination the redundant information present in the X-ray intensities of structures made up of many subunits. He solved a practical problem, namely a two-dimensional projection of the structure of the protein disk of tobacco mosaic virus, thereby pointing the way to the full solution of three-dimensional virus structures.

In the laboratory of S. C. Harrison at Harvard, he played a key role in the earlier stages of the X-ray work on tomato bushy stunt virus, among other things, using the low resolution structure reconstructed from electron micrographs to locate the heavy atoms used in the isomorphous replacement analysis.

On his return to Cambridge, Jack turned to the problem of refining the structure of the monoclinic form of tRNA, solved there earlier. This analysis, as well as settling the details of the tertiary interactions, led to the discovery of certain new correlations in nucleotide conformation and showed up the magnesium and spermine binding positions in the molecule. In an extension of this work on tRNA, Jack, together with M. Levitt, developed a particularly rapid and powerful method of refinement, which combines X-ray results and energy analysis. The method has been applied successfully to a number of proteins as well, and bids to become widely accepted.

Almost none of his friends or colleagues knew of Tony Jack's heart condition. Though quiet by nature, he was ever energetic and worked with astonishing rapidity. He was a man of varied interests. As an undergraduate he played in the 'Pineapple Truck' pop group, having built his own bass guitar. He was widely read in 18th century English literature, he collected its authors and was a stout Johnsonian. He is survived by his wife, Sharon Bellard, a chemical crystallographer, whom he met at Harvard.

A. Klug

announcements

Meetings

31 January–1 February, **Computerisation of Animal Toxicology Study Data,** Virginia (Dr Thomas Davis, Metpath Inc, 1 Malcolm Ave, Teerboro, New Jersey 07608.

25 February–2 March, **Fundamentals of Quantum Theory and Quantum Field Theory,** Palm Coast, Florida (Director, Sanibel Symposia, Williamson Hall, University of Florida, Gainesville, Florida 32611).

6–10 March, **Asteroids and Planets X,** Tuscon (T. Gerhrels, Lunar and Planetary Laboratory, University of Arizona, Tuscon, Arizona 85721).

9–10 March, **11th Stadler Genetics Symposium,** Columbia (G. P. Rédei, 117 Curtis Hall, University of Missouri, Columbia, Missouri 65211).

12–16 March, **Tunnelling '79,** London (The Institution of Mining and Metallurgy, 44 Portland Place, London W1, UK).

13 March, **Topics of Organic Chemistry —Protecting Groups,** London (Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

20 March, **Toxic Considerations in Fats and Oils Products and Derivatives,** New Jersey (George Willhite, American Oil Chemist's Society, 508 S Sixth St, Champaign, Illinois 61820).

27 March–10 April, **The Mechanism of Continental Drift and Plate Tectonics,** Newcastle (W. F. Mavor, School of Physics, The University, Newcastle upon Tyne, UK).

28–30 March, **Nuclear Structure and Elementary Particle Physics Conference,** Birmingham (Dr J. M. Nelson, Dept of Physics, University of Birmingham, PO Box 363, Birmingham, UK).

30 March, **Metal Toxicities,** Aberdeen (The Nutrition Society, Chandos House, 2 Queen Anne St, London W1, UK).

2–4 April, **Vacuum Processes for the Electronics Industry,** Sussex (Carole Meads, Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

2–6 April, **Diagnostic Methods in Clinical Virology,** Guildford (Dr S. A. Volkers, Flow Laboratories Ltd, Irvune, Ayrshire, UK).

9–11 April, **Flammability of Fibres and Textiles,** Salford (The Administrative Assistant, Room 110, Registrar's Dept, University of Salford, Salford, UK).

19 April, **Gas Injection into Liquid Metals,** Newcastle (Dr A. E. Wraith, Dept of Metallurgy & Engineering Materials, The University, Newcastle upon Tyne, UK).

19–21 April, **The Lower Swansea Valley: Retrospect and Prospect,** Swansea (Dr R. D. F. Bromley, Dept of Geography, University College of Swansea, Swansea, UK).

24 April, **Aspects of Xylem Differentiation,** Reading (Dr J. R. Barnett, Plant Science Laboratories, University of Reading, Whiteknights, Reading, Berks, UK).

24 April, **Carbohydrate Metabolism in Ruminants,** London (The Nutrition Society, Chandos House, 2 Queen Anne St, London W1, UK).

21–23 May, **4th International Symposium on Cooley's Anemia,** New York (Conference Dept, The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021).

24–27 May, **Polyploidy: Biological Relevance,** Washington (Walter H. Lewis, Dept of Biology, Washington University, St Louis, Missouri 63130).

22–25 May, **13th International Leucocyte Culture Conference,** Ottawa (K. Charbonneau, Executive Secretary, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6).

30 May–1 June, **33rd Annual Frequency Control Symposium,** New Jersey (US Army Electronic Research & Development Command, Delet -MF, Fort Monmouth, New Jersey 07703).

13–16 June, **37th Annual Meeting of the Scandinavian Radiological Society,** Stockholm (Tor Drevvatne, NR 37 c/o RESO Kongresstjänst, S-105 24 Stockholm, Sweden).

8–14 July, **11th International Congress of Biochemistry,** Toronto (K. Charbonneau, Executive Secretary, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6).

9–13 July, **5th International Workshop on Gene Mapping,** Edinburgh (Prof. H. J. Evans, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh, UK).

16–20 July, **6th International Conference on Energy Transfer,** Rodez (Mrs M. Hueyz-Aubert, Groupe de Recherche Thermiqued, Ecole Centrale, Grand Voie de Vignes, Chatenay-Malabry 92290, France).

17–23 July, **4th Annual Advanced Institute in Methods of Immunological Diagnosis,** Michigan (Noel R. Rose, Dept of Immunology and Microbiology, Wayne State University School of Medicine, 540 East Canfield, Detroit, Michigan 48201).

23–27 July, **Plant Membrane Transport Workshop,** Toronto (Dr J. Dainty, Dept of Botany, University of Toronto, Toronto, Ontario, Canada M5S 1A1).

Reports & publications

UK & Ireland–November

National Radiological Protection Board. NRPB-R82: Annual Limits of Intake of Radionuclides for Workers. By N. Adams, B. W. Hunt and J. A. Reissland. Pp.42. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1978. Obtainable from HMSO.) £1.50. [11]

Annual Report on the Meteorological Office, 1977. (Met. 0 916.) Pp.xiv+136. (London: HMSO, 1978.) £4 net. [31]

Provisional Atlas of the Mammals of the British Isles. Edited for the Mammal Society by Henry R. Arnold. Pp.13+58 maps. (Abbots Ripton, Huntingdon: Natural Environment Research Council, The Institute of Terrestrial Ecology. Monks Wood Experimental Station, 1978.) £3. [31]

Proceedings of the Royal Irish Academy. Section A: Mathematical and Physical Sciences. Vol. 78, No. 5: A Characterisation of Harmonic Functions. By A. G. O'Farrell. Pp.33–36. 84p. Vol. 78, No. 8: A Note on the $SU_n \rightarrow Sp_n$ Reduction. By G. Iommi Amunátegui. Pp.55–61. £1.10. Vol. 78, No. 9: Berberian-Quigley and the Ghost of a Spectral Mapping Theorem. By R. E. Harte. Pp.63–69. 90p. Section B: Biological, Geological and Chemical Science. Vol. 78, No. 5: An Inter-Till Fresh Water Deposit at Hollymount, Maguiresbridge, Co. Fermanagh. By A. M. McCabe, G. F. Mitchell and F. W. Shotton. Pp.77–89. £1.10. Vol. 78, No. 6: Stratigraphy, Sedimentology and Palaeontology of the Silurian Rocks of the Galty Mountain Area. By Audrey Anna Jackson. Pp.91–112+plate 1. £1.77. Vol. 78, No. 7: The Acari (Hydrachnellae) of the River Finn System and the Oweneagh River, Co. Donegal. By J. C. Conroy. Pp.113–123. £1. Vol. 78, No. 8: Litteral and Benthic Investigations on the West Coast of Ireland—VIII. (Section A, Faunistic and Ecological Studies.) A New Species of Amphipod, *Lembos denticarpus* SP. NOV. (Aoridae), from Galway Bay. By A. A. Myers and D. McGrath. Pp.125–131. 75p. Vol. 78, No. 9: "Channel" (Fragipan-Like) Horizons on the Soils of a Drumlin in North-Central Ireland. By F. McCabe, J. F. Collins and M. Walsh. Pp.133–144. £1.20. (Dublin: Royal Irish Academy, 1978.) [61]

Engineering Structures, Vol. 1, No. 1, October 1978. (The journal of Earthquake, Wind and Ocean Engineering.) Pp.1–56. Edited by Dr J. Munro and Professor P. L. Gould. Published quarterly. Annual Subscription, including postage by surface mail, £35. Single copy £8.25. (Haywards Heath, Sussex: IPC (Sales and Distribution), Ltd., Oakfield House, Perrymount Road, 1978.) [61]

Second Report from the Select Committee on Science and Technology. Session 1977–78. Innovation Research and Development in Japanese Science Based Industry. Vol. 1: Report. Pp.xl. (London: HMSO, 1978.) 90p net. [71]

Science Research Council. The Royal Greenwich Observatory, Report for 1976 October to 1977 September. Pp.51+4 plates. (Hailsham, East Sussex: Royal Greenwich Observatory, Herstmonceux Castle, 1978). £1 net. [71]

An Introduction to Cosmology. By T. R. Thomson. Pp.24. (Minety, Malmesbury, Wilts.: Taylor and Sons, 1978.) £1. [81]

A Survey of the Locations, Disposal and Prospective Uses of the Major Industrial By-Products and Waste Materials in Scotland. By E. M. Lawson and P. J. Nixon. (CP 50/78.) Pp.54. (Garston, Watford: Distribution Unit, Building Research Establishment, 1978.) [131]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 291 (First Part): The Northern Great Barrier Reef. Part A of a Discussion organised by D. R. Stoddart and Sir Maurice Yonge, FRS. Pp.1–197. (London: The Royal Society, 1978.) UK £16.80; Overseas £17.30. [141]

Potato Marketing Board. Annual Report and Accounts, 1978. Pp.48. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1978). [151]

nature CLASSIFIED ADVERTISEMENTS

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APPOINTMENTS VACANT

INTERNATIONAL LIVESTOCK CENTRE FOR AFRICA

One of the international research institutes funded by the Consultative Group on International Agricultural Research, is expanding Information and Training Services and invites applications from qualified and experienced personnel in

- **AGRICULTURE TRAINING**
- **CONFERENCE ADMINISTRATION**
- **EDITORIAL SERVICES**
- **LIBRARY MANAGEMENT**
- **HEAD OF PUBLICATIONS**
- **SCIENTIFIC TRANSLATION** (English–French)

The majority of the staff to be recruited will be based in Addis Ababa, Ethiopia, though some can expect to be based at regional centres in Nairobi, Kenya and Bamako, Mali. Some staff members are required to direct activities in the field of information and training, while others are required to fulfill specific functions within the areas listed.

Salaries will vary according to post and experience, but are generally competitive in the range US$10,000 to US$32,200 per annum. An outstanding applicant, appointed to a position of wide responsibility, could be offered a salary exceeding US$32,200 per annum. Most international staff in I.L.C.A. are required to be bilingual in English and French.

Most appointments will be made on a two-year contract, though applications from those wishing a short terms contract (6 or 12 months) are also invited.

Conditions of service include annual home leave, furnished accommodation and favourable family and cost of living allowances. International staff are accorded some diplomatic privileges (such as immunity from local taxes, etc.) enjoyed by similar international organisation in its centres.

Applications should be submitted in writing to:

The Personnel Officer
I.L.C.A.
P.O. Box 5689
ADDIS ABABA
Ethiopia

to arive before February 28 (quoting ref. ITS).

9138(A)

RESEARCH OFFICER/ TECHNICIAN

required by the Medical Research Council Unit at Hammersmith Hospital. The work will consist mainly of biochemical changes in cellular systems after exposure to radiation and other toxic hazards. Previous experience of biochemical techniques would be advantageous. Candidates should possess a degree or equivalent qualification in a relevant discipline. Salary, according to age and experience, in the range £3,615 to £5,256 (inclusive of London Weighting).

Please write, giving qualifications and curriculum vitae, to: Miss A. Pires, M.R.C. Cyclotron Unit, Hammersmith Hospital, Ducane Road, London W12 0HS.

9119(A)

McGILL UNIVERSITY POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a Postdoctoral Research Fellow to work with Dr D. G. Osmond on cellular aspects of the immune system, particularly the differentiation and potentials of lymphocyte populations produced in the bone marrow. The position is available from April 1, 1979 for up to two years post Ph.D. at a salary of $11,600 to $12,720. A background in general cytology, cellular immunology or experimental hematology is desirable. Applications, including curriculum vitae and names of two referees, should be sent to Dr D. G. Osmond, Department of Anatomy, McGill University, 3640 University Street, Montreal, PQ, Canada H3A 2B2. Telephone (514) 392-4898.

9092(A)

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF BIOCHEMISTRY

TECHNICIAN (Grade 3)

required to assist in an S.R.C. supported project for a period of three years on biochemical and biophysical aspects of plant photosynthesis. Previous laboratory experience is expected and familiarity with spectrophotometric/fluorometric instrumentation would be an advantage. The successful applicant will be able to learn a wide variety of laboratory techniques and will be encouraged to work with minimal supervision. Commencing salary £2,688 per annum (under review).

Please write giving the names of two referees, to the Administrative Officer (Personnel), (Ref. S1147/N), The University, Sheffield S10 2TN.

9105(A)

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS AL5 2JQ

SURVEY DEVELOPMENT OFFICER (ENTOMOLOGY)

required to develop ways of applying and disseminating insect survey information for pest advisory purposes. The appointee will extend the usefulness of the present Rothamsted system for national and regional monitoring/forecasting of pests, initially aphids, and promote its adoption.

The work will require the imaginative application of modern analytical and communications methods applied with a keen interest in crop protection.

Qualifications: 1st or 2(1) hons. degree in Agriculture or Applied Entomology, with at least two years' relevant experience in either agricultural data processing, economics, or communications, and/or crop protection and advisory work.

Appointment in grade of Higher Scientific Officer (£4,101 to £5,448) or Senior Scientific Officer (£5,154 to £6,898). At least four years' postgraduate research or other approved experience is required for appointment as S.S.O. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees, and quoting Ref. 384 by February 1, 1979. Further details on request.

9106(A)

The School of Medicine of the STATE UNIVERSITY OF NEW YORK at Stony Brook

is conducting a search for a Chairperson of the Department of Neurosurgery who will be responsible for developing an academic programme and a patient care programme at the School and at the University Hospital. Address inquiries to: Leonard E. Meiselas, M.D., Chairman of Search Committee, SUNY at Stony Brook, Long Island, N.Y. 11794. Equal Opportunity/Affirmative Action Employer.

9107(A)

UNIVERSITY OF THE WITWATERSRAND AND SOUTH AFRICAN INSTITUTI FOR MEDICAL RESEARCH,

Johannesburg, South Africa

CHAIR OF TROPICAL PATHOLOGY IN THE SCHOOL OF PATHOLOGY

Applications are invited for appoin ment to the above post of the join staff of the University and the Inst tute, in the School of Pathology. Dutie are to be assumed as soon as possibl

Salary attached to the appointmen will be up to a maximum of R19,20 per annum. Details are explained i the information sheet.

Intending applicants should obtai the information sheet attached to thi post from the Registrar, University c the Witwatersrand, Jan Smuts Avenu Johannesburg, 2001, South Africa, wit whom applications should be lodge not later than January 31, 1979.

9099(A)

READVERTISEMENT

Institute of Obstetrics and Gynaecology UNIVERSITY OF LONDON

Queen Charlotte's
Maternity Hospital
Goldhawk Road
London W6 0XG

Applications are invited for the post of

SCIENTIFIC PROGRAMMER

GRADE 1B

at the Institute of Obstetrics an Gynaecology, Queen Charlotte Maternity Hospital. Salary scale: £3,38 to £5,604 plus £450 London Weightin Allowance. The primary function c this post is to support a new PDP1 programmable remote-job-entry term nal connected to the University CD computer installation. The work wi involve an interesting variety of sof ware development, plus the oppo tunity of real-time programming fc mass spectrometry. The successfi applicant will work in a small, live research department with a lot c scope for inventive development.

A scientific background would t an advantage and registration for higher degree would also be a po sibility.

Further details may be obtain from the Secretary, Institute c Obstetrics and Gynaecology, Chels Hospital for Women, Dovehouse Stree London SW3 6LT.

Applications, together with cu riculum vitae and the names ar addresses of two referees should t sent to: Dr G. S. King, Chemic Pathology Department, Que Charlotte's Maternity Hospital, Gol hawk Road, London W6 0XG, t Monday, February 5, 1979.

Readvertisement is necessary due the failure of the appointed person take up the position, previous app cants who wish to be re-consider should notify Dr King. 9095(A)

UNIVERSITY OF DUBLIN
Trinity College

LECTURER IN BOTANY

Applications are invited for the above post in the Department of Botany, Trinity College, Dublin, from candidates with special interests in Taxonomy/Systematics *or* Population Ecology. A knowledge of the Irish flora and an interest in field botany in Ireland would be an advantage.

Salary Scale: £4,232 to £8,321. Appointment is likely to be made within the range £4,232 to £4,680. There is a non-contributory pension scheme.

Application forms and further particulars relating to the post may be obtained from:

The Establishment Officer
West Theatre
Trinity College
Dublin 2

to whom completed applications should be sent by not later than January 31, 1979. 9067(A)

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOLOGICAL SCIENCES

Applications for a post of

LECTURER

in the above Department are invited from bacteriologists, especially those with a broad bacteriological background and with research interests in molecular genetics.

Salary scale £3,883 to £7,754, with initial placing depedent on qualifications and experience. Superannuation under USS (or continuation of FSSU). Grant towards removal expenses to Dundee.

Applications (six copies), containing the names and addresses of three referees, should be sent by January 30, 1979, to The Secretary, The University, Dundee DD1 4HN, from whom further particulars are available. Please quote Reference EST/74/78J.

9058(A)

MONASH UNIVERSITY
Melbourne, Australia
DEPARTMENT OF PHARMACOLOGY

TUTOR/SENIOR TUTOR

Applicants should possess either a doctorate in pharmacology or physiology, or a medical degree. Preference will be given to candidates with research interests in central nervous, cardiovascular pharmacology or toxicology. The Department of Pharmacology has the responsibility for teaching pharmacology to B.Sc., B.S. (Hons); B.Med.Sci., M.B.B.S., M.Sc. and Ph.D. degrees. Research interests: Pharmacology of central and peripheral synapses; mechanisms of action of hypotensive agents; cardiovascular changes in hypertension; adrenergic mechanisms; prostaglandins; actions of drugs on memory.

Salary: Tutor $A11,220 to $A13,210 p.a.; Senior Tutor $A13,606 to $A15,586 p.a. Travelling and removal allowance. Repatriation. Assistance with temporary housing.

Enquiries about the Department to Professor A. L. A. Boura. Applicants should quote reference No. 31013. Closing date: January 31, 1979. Applications in duplicate to the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria, Australia 3168, giving age, qualifications, experience and names and addresses of three referees.

9142(A)

OVERSEAS DEVELOPMENT

KNOW-HOW: vital to developing countries

2 Experts (Resident Advisers) in Coastal Aquatic Biology

INDONESIA

At the University of Diponegoro to assist in development of courses, preparation of lectures and use of scientific equipment; to advise on suitability of local staff for UK training. Applicants should be well qualified (equivalent to UK University lecturer) with research interests in some aspect of aquatic biology, preferably marine and/or fisheries biology. Teaching experience is important and evidence of the ability to assist in the development of teaching programmes in a range of different biological topics.

Appointments 18 months initially, with possibility for extension. Salary (UK taxable) according to qualifications and experience plus variable tax free overseas allowance currently in range £1,890 to £4,080 p.a. Superannuation rights may be safeguarded. (Ref 317 X).

Cotton Entomologist

TANZANIA

Required as a part of a 7 man Cotton Research and Development Team to plan and implement the Cotton Research Programme operated from the Agricultural Research Institutes at Ukiriguru and Ilonga. The entomologist will plan and implement the cotton entomology programme. Main work is comparison of insecticides and application methods, including ultra-low volume application, for control of cotton pests, mainly American ball worm, cotton stainers and aphids. The economics of cotton spraying and an examination of the factors affecting yield responses currently being achieved by farmers are also to be studied. Applicants should possess agricultural science degree and have at least 3 to 5 years' relevant postgraduate experience.

Appointment 2 years. Salary (UK taxable) in range £4,700 to £6,500 p.a. plus a tax free overseas allowance in range £1,400 to £3,370 p.a. (Ref E331 X).

The posts are wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, children's education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, quoting reference stating posts concerned, and giving details of age, qualifications and experience to:—

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London SW1E 5DH.

ODM

9076(A)

HELPING NATIONS HELP THEMSELVES

THE OPEN UNIVERSITY
FACULTY OF SCIENCE

RESEARCH ASSISTANT IN BIOLOGY

A Research Assistant is required for a project on the development of social behaviour in marmosets. The work will involve systematic observation of the social behaviour of captive groups of marmosets, computer-based analysis of behavioural data, and assisting in the supervision of the marmoset colony. Applicants should have at least a good first degree in Biology Previous experience in ethological and/or primatological research and computer-based analysis of behavioural data would be an advantage

The appointment is for three years. Ref: 3719

RESEARCH ASSISTANT/ FELLOW IN BIOLOGY
(Drosophila Research Project)

Applications are invited for a temporary research position with the Drosophila Research Project in the Biology Discipline at the Open University. The project is to investigate the role of physiological adaptation in evolution, under the direction of Dr. Mae Wan Ho. Candidates should have a good first degree in a relevant area, and some experience in population genetics, preferably of Drosophila. A relevant PhD degree will be an advantage

This post is available initially for one year, with the possibility that the grant may be renewed. Ref: 3721

Commencing salary will be within the range £3,384 to £3,883 per annum on the 1B scale for Research and Analogous staff, with USS benefits.

PLEASE QUOTE THE REFERENCE NUMBER OF THE POST IN WHICH YOU ARE INTERESTED

Application forms and further particulars are available from The Recruitment Office (JD3719/2), The Open University, P.O Box 75, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63404: there is a 24 hour answering service on 63868.

Closing date for applications: 25 January 1979.

9103(A)

Lesotho
Forest Research Officer
Up to £8,590 plus allowances

The successful candidate will initiate and carry out research into the silvicultural problems of Lesotho. This will involve the evaluation of various methods of soil preparation, conducting trials on various species, the collection and analysis of data, and investigation of management systems.

Candidates should hold a degree in forestry and have considerable experience in forestry research.

Salary includes a substantial tax-free allowance paid under Britain's overseas aid programme. Basic salary attracts 25% tax-free gratuity.

Benefits include free passages, generous paid leave, children's holiday visit passages and education allowances, subsidised housing, appointment grant and interest-free car loan.

The terms on which civil and public servants may be released if selected for appointment will be subject to agreement with their present employers.

For full details and application form write quoting MD/129/NA or telephone 01-222 7730 ext 3603.

Crown Agents

The Crown Agents for Oversea Governments and
Administrations, Recruitment Division,
4 Millbank, London SW1P 3JD.

9071(A)

SOUTH AFRICAN ASSOCIATION FOR MARINE BIOLOGICAL RESEARCH
post of
DIRECTOR

The South African Association for Marine Biological Research invites applications from suitably qualified applicants for the post of Director

The post of Director of the Association includes the administration of the Aquarium, the Dolphinarium and the Directorship of the Oceanographic Research Institute. The Director will be a full member of the Senate of the University of Natal Appointment will be, in the first instance, for a probationary period of two years, and shall be within the salary scale R15,000 by R600 to R18,000 per annum.

Additional benefits include among others, membership of a Pension Fund and membership of a Medical Aid Scheme.

Applications for the post should be in writing, addressed to the Chairman, and should include full professional and personal particulars of the applicant, including academic qualifications and experience

Further particulars regarding this post can be obtained upon written application to the Chairman of the Association, PO Box 1726, DURBAN 4000

The closing date for receipt of applications is March 15, 1979.

9041(A)

MARINE INVERTEBRATE ZOOLOGIST & CURATOR
HARVARD UNIVERSITY

is seeking to fill a tenured professorship in marine invertebrate zoology in The Department of Biology to be held conjointly with a curatorship in The Museum of Comparative Zoology Preference will be given to applicants on the basis of (1) a strong research program in systematics, and/or comparative morphology, and/or reproductive biology of marine invertebrates (Mollusca excluded), (2) experience in curatorial management of systematic collections of marine invertebrates, and (3) ability to offer instruction in invertebrate zoology at both undergraduate and graduate levels

Applicants should send a curriculum vitae and statement of research interests to: Farish A Jenkins, Jr, Chairman, Department of Biology, The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138.

Harvard is an Equal Opportunity/ Affirmative Action Employer.

8987(A)

UNIVERSITY OF LEICESTER
DEPARTMENT OF GENETICS
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from persons with an interest in biochemistry or molecular genetics for a postdoctoral research position financed by an M.R.C. Project Grant. The project will investigate the mechanism of synthesis and assembly into membranes of specific cytoplasmic and outer membrane polypeptides in *E. coli* This will involve the use of cloned genes for membrane proteins and coupled transcription/translation from DNA templates in *in vitro* systems The work is likely to involve close collaboration with research groups in the Department studying the physiology of cell division in *E coli* and the cloning of division proteins. The appointment will be tenable for up to 3 years from February 1, 1979 or as soon after as can be arranged.

Starting salary £3,660 per annum with full superannuation provision.

Applications with names of two referees to Dr L B Holland, Department of Genetics, University of Leicester, Leicester LE1 7RH.

9120(A)

UNIVERSITY OF MELBOURNE
Australia
RESEARCH FELLOW
Grade II (Limited Tenure
in Antarctic Engineering in
Antarctic Engineering Grou
Faculty of Engineering

Applications are invited for
position of Research Fellow Grad
in the field of Antarctic Engineer
The appointment will be a lim
tenure position for three years c
mencing as early as possible a
April 1, 1979.

The Antarctic Engineering Grou
being formed within the Faculty
Engineering as part of a major
development in Antarctic Studies
the University of Melbourne. Ac
collaboration will be maintained
the Antarctic Division (Commonwe
Department of Science and Envi
ment) on all research and developm
projects.

Areas of interest within the Fac
of Engineering include the follow
dvelopment of iceberg towing
harvesting technology, containment
dispersal of oil spillage in Antar
waters, design of man-made structu
above and below the air/ice interf
Qualifications: Applicants should h
a degree in Engineering or App
Science or appropriate qualificati
and would be expected to have c
siderable professional experience
research background in specialist a
relevant to Antarctic Engineering.
Duties include: Personal research
development in one or two areas
the above mentioned research fie
coordination of activities on Antar
Engineering within the Faculty
Engineering, Collaboration on proj
associated with the Habitat Group
the Faculty of Architecture, Build
and Town and Regional Planning
Salary: $A15,179 to $A19,940
annum

Enquiries may be directed to
Dean, Faculty of Engineering,
fessor L. K. Stevens or to Dr H
Graze in the University

Further information, includ
details of application procedure
conditions of appointment, is availa
from the Registrar, University
Melbourne, Parkville, Australia, 3
or the Association of Commonwe
Universities (Appts), 36 Gor
Square, London WC1H 0PF. Appl
tions close on January 31, 1979

9123(A

MEDICAL RESEARCH COUNCIL
LABORATORY OF MOLECULAR BIOLOGY
Applications are invited from
RESEARCH SCIENTISTS
for two posts

which will become available in
Cell Biology Division of the Lab
tory of Molecular Biology from
start of the next academic year.

1. Post for research in developme
genetics of nematodes. Candid
should have research experience
genetics, especially of eukaryo
2. Post for research in biochem
mechanisms of gene expression
nematode development Candid
should have research experience
biochemistry, especially in areas
transcription and protein synthe
also knowledge of DNA clo
techniques

The appointments will be for
years with a possibility of even
tenure to retiring age. Candid
must have at least an upper sec
class honours degree and six y
relevant postgraduate experience

Salary on scale starting from £5,
per annum

Applications, with full curricul
vitae and names of two refer
should be sent to the Administra
Secretary, MRC Laboratory
Molecular Biology, The Med
School, Hills Road, Cambridge
2QH, to be received not later
February 12, 1979. 9129(

Tropical Products Institute

Pharmacologist/Toxicologist

The scientist appointed to this London based post will carry out a variety of pharmacological, toxicological and nutritional studies on renewable tropical products, using *in vitro* and *in vivo* tests and short term feeding trials. The work will involve planning experiments, administering test materials to animals or tissue cultures, assessing the effects of the experiment, determining the statistical significance of results and writing reports on the findings. In addition, the successful candidate will train visiting overseas personnel and deputise for the head of the sub-section when necessary. Weekend work, attending to care of animals, is required on a rota basis.

Candidates, normally aged under 27, must have a Degree/HND/HNC or equivalent in an appropriate subject. Possession of a Home Office Licence for working with animals or the aptitude for obtaining one is essential. The appointment calls for an ability to work with the minimum of supervision.

Appointment as Scientific Officer (£3300-£4880) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 1 February 1979) write to Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB. *Please quote ref: SA/95/JD.*

Army Blood Supply Depot, Aldershot

Scientists: Haematology & Blood Transfusion Work

These posts offer an opportunity to experienced haematologists and blood transfusion scientists to join a team working on all aspects of blood banking and blood group serology.

The senior appointment involves responsibility for the day to day running of the blood processing Laboratory where ante-natal serology and antibody identification is undertaken; the external quality assurance programme for world wide laboratories and the internal quality control programme covering haematology, bio-chemistry, bacteriology, sterility and serology. In addition the successful candidate will participate in various training courses undertaken during the year. Appointment as Senior Scientific Officer (£5150-£6900) or Higher Scientific Officer (£4100-£5450) according to qualifications and experience.

There is a second post, at Scientific Officer level, concerned with the routine processing of donor bloods, ante-natal serology and identification of antibodies. The person appointed will also prepare and issue blood products, help with research projects and supervise the work of junior staff. Salary is in the range £2840-£4410 with starting salary according to qualifications and experience.

Candidates, normally aged under 32, should have a Degree/HND/HNC or equivalent in an appropriate scientific subject. Some experience in haematology and blood transfusion is essential; for the senior appointment at least 2 years' relevant postgraduate experience is required.

The successful candidates will be given every encouragement to study for a higher qualification. There are promotion prospects and a non-contributory pension scheme.

For further details and an application form (to be returned by 6th February 1979) write to Ministry of Defence, CM(S)1b3, Room 310, Savoy Hill House, Strand, London WC2R 0BX. *Please quote ref: SA/2/FYA.*

ALL THESE POSTS ARE OPEN TO MEN AND WOMEN. 9050(A)

Science Group
CIVIL SERVICE

NEUROBIOLOGY AND BEHAVIOUR

A position at the Assistant Professor el is available in the Biology partment, Princeton University. culty in the Neurobiology and Be- iour area include M. Constantine- on, H. Horn, A. Gelperin, J. uld, R. Lisk and W. Quinn. idence of exceptional promise in earch is essential. Applicants should d curriculum vitae, statement of earch experience and three letters recommendation to: Dr Alan lperin, Neurobiology Search Com- tee, Department of Biology, nceton University, Princeton, N.J. 40. Princeton University is an ıal Opportunity/Affirmative Action ployer. 9112(A)

THE UNIVERSITY OF MANCHESTER RESEARCH ASSOCIATE IN MEDICAL BIOPHYSICS

Applications are invited from engineers or physical scientists who wish to join a small interdisciplinary group working on the important problem of incontinence. The appointee would be expected to take a particular interest in models of the bladder and urethra and to apply this work to the design of artificial drainage systems for the management of incontinence. The appointment is for up to 3 years. Starting salary range p.a.: £3,883 to £4,882. Further information from Professor B. R. Pullan, Department of Medical Biophysics, Stopford Building, University of Manchester, Manchester M13 9PT. 9045(A)

ASSISTANT PROFESSOR

YALE UNIVERSITY

DEPARTMENT OF BIOLOGY

This Department invites applications for appointment beginning in academic year 1979–80 or 1980–81 as Assistant Professor from persons with interests in molecular aspects of Genetics or Development. The appointee will be expected to contribute to a strong program of research and scholarship in Molecular Biology, and to participate in undergraduate and graduate teaching.

Send curriculum vitae, resume of past and present research, and names of three referees to:

Chairman
Department of Biology
Yale University
New Haven, Conn. 06520

Yale University is an Equal Opportunity/Affirmative Action Employer.
9132(A)

Director General Nature Conservancy Council

The Council seek a Director General to succeed Mr R E Boote, CVO, who reaches retiring age in Spring 1980

The appointment is a statutory one under the Nature Conservancy Council Act, 1973, made by the Council with the approval of the Secretary of State for the Environment.

The Director General is responsible to Council for the direction and organisation of the NCC, for advice on the formulation of Council's policies and for their implementation, and has personal responsibilities for effective management and financial control as the Accounting Officer He is assisted by a Deputy Director General and Chief Scientist, and by Directors for England, Scotland and Wales, to whom there is considerable delegation.

The NCC is an Independent body set up under the 1973 Act It is the statutory adviser to Government on nature conservation in Great Britain and is involved with a wide range of interests at home and abroad

In particular, the NCC is responsible for 163 National Nature Reserves and has responsibilities in relation to nearly 4,000 Sites of Special Scientific Interest It commissions and undertakes research and makes grants to many voluntary bodies Its annual budget is around £7m. and it employs 650 full time staff, of whom half are scientists, land agents and wardens

The post is open to a person with a good knowledge and background in, for example, biological science, land use (such as agriculture or forestry), land management or other related disciplines together with an understanding and knowledge of environmental issues within the legislative, planning and educational framework The successful candidate must be able to demonstrate leadership in promoting nature conservation and liaising with a variety of organisations both governmental and voluntary and to operate both nationally and internationally in a wide range of public and professional forums. He or she must have the capacity and experience in management and administration to direct effectively an organisation operating throughout Great Britain using a variety of scientific, land management and administrative skills

The post is also open to candidates from within the Nature Conservancy Council and the Public Service

The salary at April 1, 1980 will be £16,000 per annum, plus London weighting The NCC has its own non-contributory pension scheme based on that of the Civil Service. The post is based at NCC's Great Britain Headquarters in London.

Applications giving career details, including present salary and responsibilities, with information of special relevance to the post, should be sent, envelope marked *DG Post* and *Personal*, to Professor F G T Holliday, CBE FRSE, Chairman, Nature Conservancy Council, 19/20 Belgrave Square, London SW1X 8PY by not later than Friday March 9, 1979. In the first instance two referees should be named. Interviews will take place in May and June 1979 and the successful candidate will be expected to take up post early in 1980

9068(A)

GREATER GLASGOW HEALTH BOARD
EASTERN DISTRICT
SCOTTISH ANTIBODY PRODUCTION UNIT

SENIOR BIOCHEMIST

Applications are invited from suitably qualified persons for the post of Senior Biochemist in the Scottish Antibody Production Unit (SAPU) based at the Radioimmunoassay Unit, Department of Biochemistry, Royal Infirmary, Glasgow The SAPU is an interdisciplinary group with constituent components based at the West of Scotland Regional Blood Transfusion Centre, Law Hospital and Departments of the University of Glasgow. The group is involved in the development of antisera and related products for diagnostic use in hospitals throughout Scotland Experience in immunoassay and immunochemical techniques would be an advantage for the present post

Salary is in the range of £5,451 to £6,837 Conditions of employment are in accordance with the appropriate Whitley Council regulations

Candidates are invited to visit the laboratories by arrangement with Dr J. G Ratcliffe, Department of Biochemistry, Royal Infirmary, Glasgow (Tel. 041-552 3535, extension 5275)

Applications, including curriculum vitae and names of two referees, should be sent to the District Administrator, Eastern District, Greater Glasgow Health Board, 13 Bath Street, Glasgow G2 1JB, within two weeks of the appearance of this advertisement. 9062(A)

QUEENSLAND INSTITUTE OF TECHNOLOGY
Brisbane, Australia
LECTURER IN HISTOTECHNOLOGY/ HISTOCHEMISTRY

A Lecturer is required within the Department of Medical Technology to provide academic leadership in the continuing design, development and presentation of courses in Histotechnology and Histochemistry, and to maintain strong links with the histopathology segment of the medical laboratory science profession The Department offers courses in Histotechnology and Histochemistry at degree level in the B App.Sc. Medical Technology and at technician level in the Associate Diploma in Clinical Laboratory Techniques and provides opportunity for Postgraduate students in the area through the Masters Degree by Research and Thesis

Applicants should hold an appropriate higher qualification and have a record of academic and work experience at a senior level Previous teaching experience would be an advantage

Salary: Lecturer I $AUS17,764 to $AUS19,940, Lecturer II $AUS15,17 to $AUS17,357 p a

Further information may be obtained from the Personnel Officer, Q I T., G P.O. Box 2434, Brisbane Q. 4001 Australia, or from the Head of Department, Dr A J Webber, telephone (07) 221 2411 ext 554.

Applications quoting reference V 161/78, together with full details and telephone number and the names of two referees should reach the Personnel Officer by February 28, 1979

9053(A)

THE UNIVERSITY OF BRITISH COLUMBIA
DEPARTMENT OF ZOOLOGY

Applications are invited for three positions at the Assistant Professor level to teach

(1) Parasitology
(2) Introductory Biology
(3) Neurophysiology

Qualifications include a Ph D , with demonstrated ability in effective teaching the appointee for Introductory Biology must be capable of making a positive contribution to teaching large first year classes. The successful applicants will be expected to develop strong research programmes Upper level undergraduate teaching, and graduate student teaching and supervision are required.

Salary will be commensurate with experience and duties

Curriculum vitae and the names of three references should be sent to Dr G G E Scudder, Head, Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, B C V6T 1W5 Appointment effective July 1, 1979 Closing date when position filled

9087(A)

FACULTY POSITION IN ATMOSPHERIC SCIENCES

The Department of Atmospheric Sciences of the University of California, Los Angelles, has two ladder faculty positions open, starting July 1, 1979 Candidates must have a Ph D. degree in atmospheric sciences or in a related area Preferred areas of research specialisation are

1 Cloud dynamics, mesoscale dynamics, and cloud microphysics.
2 Atmospeheric chemistry or physical meteorology

Exceptionally qualified candidates in other areas of atmospheric sciences are also encouraged to apply.

Besides carrying out research in his own area of specialisation, the candidate will be expected to teach both graduate and undergraduate courses in atmospheric sciences Applicants should submit resumes, including a list of publications and the names of three references to: Chairman, Department of Atmospheric Sciences, University of California, Los Angeles, California 90024 U C L A is desirous of receiving applications from minorities and women candidates as well as from other qualified persons U C L A is an Equal Opportunity/Affirmative Action Employer. 9088(A)

COMMONWEALTH AGRICULTURAL BUREAUX
Vacancy for
SCIENTIFIC INFORMATION OFFICER
at the
Commonwealth Bureau of Soils
Rothamsted Experimental Station
Harpenden, Herts AL5 2JQ

Duties of post Preparation of abstracts for publication in *Soils an Fertilisers* and dealing with technical enquiries

Qualifications Applicants should b graduates in soil science or agricultural science Ability to read foreign languages other than French will count as an additional qualification

Salary In the scale £2,839 to £5,448 plus a compensatory allowance (taxable but not superannuable) of 3.3 per cent of basic salary to offset personal contribution to superannuation scheme. Starting salary according t qualifications and experience. Promotion to higher scales on merit

Application forms and full particulars from Executive Director Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal Slough SL2 3BN.

Closing date for applications February 15, 1979 9089(A)

MARINE BIOLOGISTS
(2 posts)
SOUTH AFRICAN MUSEUM
Cape Town

Applications are invited for the pos of Professional Officer/Senior Professional Officer on the salary scale R3,930 by R180 to R5,010 by R270 t R6,090/R6,090 by R270 to R7,440 b R390 to R8,220.

Minimum qualification: M.Sc. i Zoology, Taxonomy or Biological Oceanography Starting salary will b determined on the basis of previou experience and qualifications

The successful candidate will be required to undertake taxonomic researc on marine invertebrates, particularly in one of the following groups· Crustacea marine Mollusca, Gorgonacea Polyzoa Previous experience in the chose group will be a recommendation bu not a necessity.

Closing date: March 31, 1979.

Application forms obtainable fro the Director, South African Museum P O Box 61, Cape Town 8000, Republic of South Africa 9061(A)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF BACTERIOLOGY
POSTDOCTORAL FELLOW/ RESEARCH ASSOCIATE

required to work on a Cancer Research Campaign sponsored project i tumour immunology. Experience i working with tumours or in the fiel of cellular immunology would b desirable. Salary on National Rang IA.

Send c.v. to Dr W. H McBride Department of Bacteriology, Edinburgh University Medical School Teviot Place, Edinburgh EH8 9AG Scotland Please quote Reference 5058

9090(A)

SUNDERLAND POLYTECHNIC
FACULTY OF SCIENCE
SCHOOL OF PHARMACY
LECTURER II IN PHARMACOLOGY

Candidates should be graduates i Pharmacy, Pharmacology or Phys ology with teaching or research o other relevant experience. The lev of appointment will depend on qual fications and experience An intere in research is desirable

Salary scale· Lecturer II £4,101 t £6,051 Bar to £6,558.

Further particulars and an applic tion form may be obtained from th Personnel Officer, Sunderland Pol technic, Chester Road, Sunderlan SR1 3SD, and should be returned soon as possible. 9079(A)

LOUGHBOROUGH UNIVERSITY OF TECHNOLOGY

CHAIR IN PHYSICAL CHEMISTRY

Applications are invited from candidates with strong teaching experience and established research attainment for a Chair in Physical Chemistry in the Department of Chemistry Any specialist interest within the field of physical chemistry would be acceptable, a good publication record, research interests and the ability to lead a research group is essential.

Salary within professorial range.

Further particulars and application form from Paul Johnson, Establishment Officer, Loughborough, Leicestershire. Ref 78/58CM. 9065(A)

FACULTY POSITION IN THE DEPARTMENT OF ASTRONOMY, UCLA

The Department of Astronomy invites applications for a regular faculty osition beginning Fall, 1979. The itial level of this position is open nd dependent on the qualifications of e applicant.

Applicants with an instrumental/bservational background (including pace astronomy) are preferred; but applicants from all fields of astronomy will be considered.

A list of publications, copies of reprints, three letters of recommendation, and a statement of rearch interests should be submitted support of the application.

Deadline for application is March . 1979. UCLA is an Equal Opportunity/Affirmative Action employer.

Mail applications to:

Dr Roger K. Ulrich, Chairman,
University of California, Los Angeles,
405 Hilgard Avenue,
Los Angeles, California 90024

9086(A)

Royal Military College of Science

ECTURER IN CHEMISTRY

CHEMISTRY AND METALLURGY DEPARTMENT

The Chemist/Materials Scientist pointed will join the academic turing staff of the Royal Military llege of Science, at Shrivenham. asantly situated 7 miles outside ndon, the College has a long ucational history in chemistry, ich is currently taught at underaduate and postgraduate level to th service men and women, and also a growing number of civilian dents. The teaching emphasis of is post will be on organic chemistry, luding the chemistry of plastics, lymers and explosives, in addition, will involve responsibility for tiating a programme of relevant damental research There are exllent laboratories for teaching and search.

Candidates should have a good nours degree in chemistry or terials science, and, preferably, a D Teaching and/or research exrience advantageous Appointment ll be as Lecturer (£3,165 to £4,415) Senior Lecturer (£5,150 to £6,895) cording to age, qualifications and exrience Promotion prospects. Non-tributory pension scheme

or further details and an applican form (to be returned by February 1979) write to Civil Service Comsion, Alencon Link, Basingstoke, nts, RG21 1JB, or telephone ingstoke (0256) 68551 (answering vice operates outside office hours) *ase quote ref S/9979/3.*

9078(A)

Assistant Production Manager

Animal Health Laboratories

To join our management team at a high level and to assist the production manager in our recently established bacterial vaccines production unit at Milton Keynes He/she will be expected to function in the area of large scale antigen production and final product preparations. The person we look for will have at least a B.Sc. in microbiology/bacteriology and some years experience in biological production along with a proven record in man management.

Hoechst UK is part of a world wide organisation in which salaries and conditions are of the high order which one would expect Other benefits of employment include minimum of four weeks holiday, contributory pension scheme with free life insurance, free membership of P.P P , flexible working hours, staff restaurant with meal vouchers, sports and social club and contribution to removal expenses where appropriate.

Please write or telephone for an application form to·
Mrs. S. Matthews, Hoechst UK Ltd.,
Walton Manor, Walton, Milton Keynes, Bucks.
Telephone: Pineham 5068.

9057(A)

Hoechst

THE UNIVERSITY OF PAPUA NEW GUINEA

(Port Moresby)

Applications are invited for the post of

TUTOR/SENIOR TUTOR IN PLANT BIOLOGY

in the Biology Department

Applicants should have a commitment to innovative teaching in introductory biology courses and more advanced tropical plant biology courses, especially aspects of functional plant structure or tropical plant systematics It is hoped that the appointee would be able to take up duties at the latest in June, 1979 Interested persons can write to the Chairman, Biology Department, for further details.

Salary scales· Senior Tutor Grade I, K9,048 to K10,168 p a ; Tutor, K7,323 to K8,382 p a In addition, an allowance of K1,300 p a. if single is payable. An extra K1,000 Marriage Allowance and K156 per child Child Allowance may also be payable (£1 sterling=K1.38)

Applications should include detailed curriculum vitae, a recent small photograph and the names and addresses of three referees. Conditions of appointment, which are currently under review, include provision of housing and annual leave fares, and are available from the Secretary, Box 4820, University P.O , Papua New Guinea, with whom applications close on January 31, 1979. Applicants resident in the U K should also send one copy of application to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars are also obtainable from this address.

9125(A)

Microbiologists

Petrolite Ltd, a leading service company to the oil industry are offering an exciting opportunity for a fully qualified Microbiologist to join their Technical Services Team.

Although based in Liverpool the position will involve trouble-shooting travelling as necessary to the major oil-fields of the world in order to solve the problems caused by bacterial growth in oil-field and industrial waters Your priority will be to make full recommendations, based on practical work in the field, of specific bio-cides to be used.

To cope with the demands of this challenging position you will have Post Graduate experience in Microbiology of Corrosion plus the personal qualities of self-motivation, flexibility and initiative

An attractive salary according to experience will be negotiated with the successful candidate.

Contact Chris Bainton
01-235-7030 ext 239.

9064(A)

PER
Professional
& Executive
Recruitment

Applications are welcome from both men and women.

Centre for Overseas Pest Research, London

Scientific Librarian

The Centre for Overseas Pest Research helps governments of overseas territories, in particular the less developed countries, to solve pest problems in the fields of pre-harvest agriculture and public health through the technical co-operation programme. The Centre also provides scientific information, advice and training in Britain and overseas.

This post is in the Library Service where the person appointed will take responsibility for periodical literature and exchange material, liaison with overseas and other organizations regarding exchange of publications, and the supervision of a part-time assistant. In addition the successful candidate will scan, procure, catalogue, classify and index world-wide scientific literature including foreign language material, maintain collections of trade literature on pesticide chemicals and pesticide application equipment, deal with readers' enquiries for staff and visitors and undertake additional duties, as and when required including interpretative work in the subject field.

Candidates, normally aged under 27, must have a good honours degree, preferably in a biological science or agriculture or closely related subject, coupled with postgraduate training/experience in librarianship. An aptitude for languages is desirable.

Appointment as Scientific Officer (£3300-£4880) with starting salary according to qualifications and experience. Non-contributory pension scheme.

For further details and an application form (to be returned by 5 February 1979) write to Miss M. Cole, Centre for Overseas Pest Research, College House, Wrights Lane, London W8 5SJ. *Please quote ref: SA/50/JA.*

9077(A)

Science Group
CIVIL SERVICE

Biometrician

The Forestry Commission offers a period appointment of 3 years, for a Biometrician to join a team in the Statistics on Computing Section at the Forest Research Station, Roslin near Edinburgh.

The successful candidate will be required to help in the organisation and provision of advice to other branches of Forest Research, to advise project leaders on design of experiments and surveys, analyse results and report clearly and program various computers using remote terminal or batch. There will also be opportunities to initiate and lead practical research.

Candidates should have a good honours degree or equivalent qualifications in Statistics or related subject, plus at least 4 years relevant experience and be in the age range of 25 to 30 years on the 31 December 1978. The successful candidate will be appointed to either the Higher Scientific Officer (£4,101—£5,448) or Senior Scientific Officer (£5,154—£6,898) grade according to age, experience and qualifications.

Application forms and further details may be obtained from Mr A J Freeman, Personnel Branch, Forestry Commission, 231 Corstorphine Road, Edinburgh, EH12 7AT. Completed application forms must be returned not later than 6th February 1979. 9069(A)

IMMUNOCHEMIST

Postdoctoral position is available immediately to work on anti-tumour resistance induced by bacterial products. Strong background in chemistry is essential. Research assistant position on the same project is also available immediately. Submit resume, three references and transcripts to Professor A. Nowotny, University of Pennsylvania, Building A2, 4010 Locust Street, Philadelphia, PA 19104.

9114(A)

MUSEUM DIRECTOR

The University of Colorado at Boulder invites applications and nominations for the Director of the University Museum. The Museum conducts regional, national, and international research and educational programmes in the fields of natural history including anthropology and interacts with related University academic departments. Candidates must excel in administration, leadership, and research (including curation); must have a Ph.D.; must be strongly committed to University and community service and education; and must be able to interact positively with University administration, related academic departments, professional organisations, and funding agencies. Applications consisting of Curriculum Vitae, five names of references with addresses, and summary of administrative and research experience should be sent to:

Dr Robert A. Bye, Jr.
Chairman,
Museum Director Search Committee,
Department of Environmental,
Population and Organismic Biology,
Box 334,
University of Colorado,
Boulder, Colorado 80309 U.S.A.

Deadline for applications: March 15, 1979.

Anticipated starting date: June 1979 (negotiable).

An Equal Opportunity/Affirmative Action Employer. 9116(A)

EUROPEAN MOLECULAR BIOLOGY LABORATORY

ELECTRON MICROSCOPY TECHNICIAN

The European Molecular Biology Laboratory at Heidelberg, F.R.G. requires an electron microscopy technician to work on central nervous tissue for a project investigating visual structures in insects. Proficiency in electronmicroscopy techniques and the operation of an electronmicroscope is essential. Previous work on central or peripheral nervous system would be desirable, but not essential. The post is available immediately.

Apply to Dr N. Strausfeld, EMBL, Postfach 10.2209, D6900 Heidelberg, telephone number 06221-387-295, citing ref. 79/2. 9118(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF BIOCHEMISTRY

A vacancy exists for a Research Fellow (Range IA) who must be a postdoctoral biochemist with experience of radiochemical techniques to work for three years with Dr D. E. Briggs on an S.R.C.-funded project on the mechanism(s) regulating gibberellin release from barley embryos. The salary will be in the range £4,382 to £4,882 per annum (plus U.S.S.). The successful applicant would be required to take up appointment in the period between May and October 1979.

Applications (two copies) including a curriculum vitae and names of two referees should be sent to Miss L. R. Heyes, Senate Registry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, from whom further particulars may be obtained. The closing date is February 2, 1979.

9117(A)

KING'S COLLEGE HOSPITA
MEDICAL SCHOOL

(University of London)
Denmark Hill
London SE5 8RX

LIVER UNIT

Applications are invited for th position of Research Assistant/Fello in this busy multi-disciplinary uni from candidates with experience i cell membrane biology, immuno chemistry or protein biochemistry, t work on human liver cell surfac antigens.

Salary will be on University o London scales, according to qualifica tions and experience.

Please apply, giving full curriculu vitae and the names and addresses o two referees to the Secretary of th Medical School at the above address from whom further details may b obtained. Closing date February 1979. 9074(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL

DEPARTMENT OF ANAESTHETICS

TECHNICIAN

with H.N.C. or similar qualificatio required for this very active researc department. The person appointed wil be expected to be capable of assumin responsibility for cardio-respirator and blood gas measurements. Experi ence in gas chromatography, physic logical measurement or electronics i desirable. Starting salary within th range £4,009 to £4,854 depending o experience and qualifications, goo holidays and contributory superannu ation scheme.

Application forms may be obtaine from the Personnel Office, Royal Pos graduate Medical School, 150 Du Can Road, London W12 0HS quotin reference number 14/104/ .

9073(A)

CHELSEA COLLEGE

University of London

BASIC MEDICAL SCIENCES GROUP

Applications are invited for the posts of:—

1. POSTDOCTORAL RESEARCH ASSISTANT

(salary £4,583 to £5,081, inclusive of London Allowance

2. RESEARCH TECHNICIA GRADE 5

(salary £3,651 to £3,858, inclusive of London Allowance

to work on a three-year M.R. funded project concerned with th application and production of mon clonal antibodies to cytoskeletal pr teins from nervous tissue, in pa ticular, neurofilaments. Experience i protein chemistry, or tissue culture o immunology would be desirable.

Further particulars and applicatio with curriculum vitae to Dr B. Anderton, Basic Medical Scienc Group, Chelsea College, Manres Road, LONDON SW3 6LX.

9072(A)

POSTDOCTORAL POSITIO

of 2-year duration

available immediately for sequence a affinity labelling studies on ribulosebi phosphate carboxylase at salary $12,000 for first year. Please se resume and letters of references t Dr Fred C. Hartman, Oak Ridg University of Tennessee Gradua School of Biomedical Sciences, P. Box Y, Oak Ridge, TN 37830. *Equal Opportunity/Affirmative Acti Employer.* 9085(A)

UNIVERSITY OF STRATHCLYDE

Applications are invited for

TWO LECTURESHIPS

in the

DEPARTMENT OF PURE AND APPLIED CHEMISTRY

Applicants for one of the posts should be experienced in (experimental) INORGANIC CHEMISTRY and for the other in CHEMICAL TECHNOLOGY.

Salary scale £3,883 to £7,754 with placing according to qualifications and experience. Superannuation benefit.

Application forms and further particulars (quoting 1/79 for the Lectureship in Inorganic Chemistry and 2/79 for the Lectureship in Chemical Technology), and enclosing a self-addressed envelope (9"×4"), may be obtained from the Academic Appointments Officer, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, with whom applications should be lodged by February 26, 1979.

9059(A)

UNIVERSITY COLLEGE OF NORTH WALES

BIOCHEMISTRY

Applications are invited for the post of

PROFESSOR

of Biochemistry and Head of Department

The appointment will be from OCTOBER 1, 1979 (or a date to be arranged) and the salary will be in the professorial range.

Further particulars can be obtained from the Secretary and Registrar, University College of North Wales, Bangor, Gwynedd LL57 2DG.

Ten copies of the application (one from overseas candidates) together with the name of three referees should be sent to reach the Secretary and Registrar by Friday, February 16, 1979.

9048(A)

ISRAEL INSTITUTE OF TECHNOLOGY, HAIFA RESEARCH ASSOCIATE POSITION

available for a period of two or three years.

Experimental background in Chemical Physics, Electrochemistry and/or Surface Science required.

Contact Prof D. S. Tannhauser, Department of Physics. 9051(A)

UNIVERSITY OF CAPE TOWN

SENIOR LECTURESHIP IN INORGANIC CHEMISTRY

Applications are invited for the above post, vacant as from July 1, 1979. Appointment, according to qualifications and experience, will be made on the salary scale R8,460 by R360 to R9,900 by R450 to R11,250 per annum, plus a pensionable allowance of 19.9 per cent of basic salary.

Applicants should submit a curriculum vitae, stating present salary, research interests and publications, the date duty could be assumed, and the names and addresses of three referees.

Memoranda concerning the position and general conditions of service should be obtained from the Registrar, Room 10, University of Cape Town, Private Bag, Rondebosch 7700, South Africa, by whom applications must be received not later than April 30, 1979.

Appointment will be subject to a satisfactory medical examination.

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further information on the implementation of this policy is obtainable from the Registrar.

9094(A)

The Daresbury Laboratory has vacancies for

THEORETICIANS

in all three fields of interest of the Theory Group at Daresbury which are solid state, atomic and molecular, and nuclear.

At the laboratory a purpose-built X-ray and ultra-violet source and a major nuclear structure facility based on a 30 MV tandem Van de Graaff are under construction. An IBM 370/165 computer is installed at the laboratory, and a computational science group has recently been established working mainly in the quantum chemistry area.

Appointments will be made at salaries between £3695 and £6216, the level at which an associate is appointed depending on age, ability and experience. Posts are normally available for a fixed term of 3 years and are superannuable.

The successful applicants will be expected to take a strong interest in the experiments which are being planned on the new facilities.

Applicants, of either sex, should possess a Ph.D. degree in the relevant area, or expect to obtain one during 1979.

Closing date: 14 February 1979.

For further information please write or telephone the head of the theory group, Dr. J. B. Pendry (Tel. No. (0925) 65000 Extension 223). Applications should be sent together with curriculum vitae and the addresses of two referees, quoting reference number DL/650/9070(A) to:

The Personnel Officer

DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

9070(A)

KENYATTA UNIVERSITY COLLEGE—KENYA

(a constituent college of the University of Nairobi)

Applications are invited for

LECTURESHIPS IN THE PHYSICS DEPARTMENT

Applicants should be holders of a Ph.D. Appointees will join the staff of Physics Department offering courses to degree level to future Secondary School teachers together with an M.Sc. Physics programme. The department has special interests in teaching and in the field of Appropriate Technology and Electronics Applications. Any speciality will be considered.

Salary scale: K£2,016 to K£3,312 p.a. (K£1=£1.34 sterling.) The British Government may supplement salary in range £4,278 to £4,818 p.a. (sterling) for married appointee or £2,730 to £3,156 p.a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; subsidised housing; S.S.S.F. or F.S.S.U.; non-contributory medical aid scheme.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Registrar, Kenyatta University College, P.O. Box 43844, Nairobi, Kenya, by February 2, 1979. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9122(A)

SOUTH GLAMORGAN HEALTH AUTHORITY (T)

CARDIFF ROYAL INFIRMARY

SUPRA-REGIONAL PROTEIN REFERENCE UNIT

Applications are invited for the post of

RESEARCH ASSISTANT

to work on the development and assessment of immune complex assays in clinical chemistry. This project is supported by the Welsh Office scheme for the development of Health and Social Research. The post will be for **one year** in the first instance but renewal for the second year may be possible.

Candidates should have a degree in Biochemistry, Biological Sciences or equivalent and salary scales will be according to qualification and experience, but will be on the Whitley Scale for probationary grade Hospital Biochemists.

Further details of the project and the post can be obtained from—Dr R. Fifield, Protein Reference Unit, Cardiff Royal Infirmary, telephone 0222 492233, ext. 559.

Application forms can be obtained from Assistant Unit Administrator, Cardiff Royal Infirmary. Tel: Cardiff 492233.

Closing Date: January 26, 1979. 9081(A)

UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL

Applications are invited for the post of Technician who will work on a study of trace element metabolism in preterm infants. The job involves work in the laboratory and in the Premature Baby Unit. Familiarity with atomic absorption spectrophotometry desirable but not essential. Please submit Curriculum Vitae and the names and telephone numbers of two referees to the Secretary, University College Hospital Medical School, University Street, London WC1E 6JJ, quoting reference T/JS. 9075(A)

SURFACE PHYSICIST/ CHEMIST

Fusion energy research on plasma-wall interaction phenomenon involving atomic hydrogen chemical sputtering and surface cleaning. Uhv and surface physics/chemistry experience appropriate. Appoint at Research Associate or Senior Research Associate level. Appointment for three years initially. Well-qualified scientist sought. Appropriate salary available.

Write: Professor P. C. Stangeby, University of Toronto, Institute for Aerospace Studies, 4925 Dufferin Street, Downsview, Ontario M3H 5T6. (416) 667-7729. 9083(A)

CHEMICAL ENGINEERING RESEARCH GROUP—CSIR

Would you like an opportunity to apply your professional skills in depth to the solution of problems of an applied or fundamental nature in a stimulating interdisciplinary environment?

The Chemical Engineering Research Group—CSIR is one of over a dozen institutes of the Council for Scientific and Industrial Research. The Group is engaged in R & D and consulting services related to the utilisation of the natural resources of the country and the development of its process industry. We have openings for

SENIOR RESEARCH STAFF and temporary RESEARCH FELLOWS

PARTICLE TECHNOLOGY — FLUID DYNAMICS

Solid-fuel separation and suspension flow in process equipment requiring the application of engineering science in the processing of particulate matter.

RHEOLOGY

Investigations of the rheological properties of materials used in the process industry, particularly suspensions of particles.

HETEROGENEOUS CATALYSIS

The development of catalysts and catalytic processes for the conversion of coal and oil to liquid fuels and petrochemicals, including the downstream processing of the intermediates.

COMBINED HEAT AND MASS TRANSFER

The development of techniques to determine optimal drying procedures and the consequent selection of drying equipment for industry.

ELECTROSTATIC PRECIPITATION

Investigations leading to improvements in the control of emissions from power stations.

General requirements: a PhD or at least an advanced degree in a relevant discipline, research ability, experience in the indicated field and the ability to communicate research results.

SOME POSTS AT GRADUATE LEVEL ARE ALSO AVAILABLE

Pursuit of an advanced degree is encouraged and financially supported.

Conditions of service: Attractive remuneration, a pleasant climate and working environment on a large campus where most of the CSIR institutes are housed, scope for initiative, advance on merit, generous leave allocation, substantial housing subsidy, holiday bonus, pension and medical aid schemes, group life insurance and other fringe benefits, outstanding laboratory, workshop computing and information facilities, relocation expenses.

Apply to: The Head, CERG—CSIR, P.O. Box 395, PRETORIA, 0001 South Africa 9060(A)

Lecturer Grade II/ Senior Lecturer in Physiology

(£4101 - £7065 (Bar) - £7572)

Candidates should preferably have experience in Histology, Electron Microscopy, exercise physiology or sports science.

Further details and form of application available from The Assistant Director and Chief Administrative Officer, Trent Polytechnic, Burton Street, Nottingham. A self-addressed foolscap envelope will assist in the speedy despatch of documents.

Applications to be returned as soon as possible.

9102(A)

TRENT
POLYTECHNIC
NOTTINGHAM

UNIVERSITY OF DURHAM

DEPARTMENT OF CHEMISTRY

Applications are invited for chemists or chemical physicists for an S.R.C. Postdoctoral Senior Research Assistantship for 17 months from as soon as possible to work in collaboraton with Dr J. Yarwood on the interaction and dynamics of hydrogen bonded systems in the liquid phase. These studies which include infrared, far-infrared, Raman and neutron scattering studies are also supported by a N.A.T.O. research grant and would be carried out at Durham, the Technical University, Braunschweig and at A.E.R.E. Harwell. Some experience in one branch of molecular spectroscopy is essential and an interest in the chemical physics of molecular motions and interactions in the liquid phase would be an advantage.

Initial salary £3,883 or £4,133 per annum on National Research Range 1A plus superannuation.

Applications (three copies) naming three academic referees should be sent by January 22, 1979, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE.

8904(A)

UNIVERSITY OF DUBLIN
Trinity College

LECTURER IN GEOLOGY

Applications are invited for the above post in the Department of Geology, Trinity College, Dublin, from candidates with strong academic qualifications and with substantial interest in developing courses in applied aspects of the geological sciences, such as environmental geology and engineering geology.

Salary Scale: £4,232 to £8,321. Appointment will be made within the range £4,232 to £4,680. There is a non-contributory pension scheme.

Application forms and further particulars may be obtained from:

The Establishment Officer
West Theatre
Trinity College
Dublin 2

to whom completed applications should be returned not later than January 31, 1979. 9066(A)

DISTINGUISHED ENERGY PROFESSORSHIP AT SYRACUSE UNIVERSITY

Nominations and applications are invited for the Niagara Mohawk Energy Chair at Syracuse University. The holder of the Chair must be a recognised scholar and is expected to provide leadership in the establishment of an interdisciplinary centre of excellence for energy research and in the development of energy curricula. The candidate should have experience in high-efficiency, bulk-energy conversion techniques using environmentally acceptable processes for the utilisation of coal or nuclear fuels. The Chair is expected to be attached to the Department of Chemical Engineering and Materials Science which has a particular interest in fluidised bed technology. Outstanding scholars in other energy-related areas are also eligible. Nominations and applications should be sent to Professor David K. Cheng, Chairman, Energy Professor Search Committee, Link Hall, Syracuse University, Syracuse, N.Y. 13210, before April 1, 1979. Syracuse University is an equal opportunity affirmative action employer. 9115(A)

ANIMAL HOUSE TECHNICIAN

(Readvertisement)

Applications are invited for the pos of Technician in the School of Biological Sciences to take responsibilit for the running of an Animal Uni housing small mammals, insects an amphibia.

Candidates must have had severa years experience of Animal Hous work and hold qualifications of th Institute of Animal Technicians.

Salary on the scale £3,186 to £3,72 (under review).

Further particulars and applicatio forms may be obtained from th Superintendent of Laboratories, Schoo of Biological Sciences, University o East Anglia, Norwich NR4 7TJ, t whom they should be returned withi two weeks of the publication of thi advertisement. 9134(A)

POSTDOCTORAL POSITION

Ph.D. of M.D. with good background in membrane biochemistry fo studies of composition and structur of nexuses (gap junctions). Laborator is well equipped for membrane bio chemistry and ultrastructure (includin freeze-fracture, rapid freezing, lase diffraction etc.). Starting date, Marc 1, 1979. Send resume and two letter of recommendation to: Camill Peracchia, Department of Physiolog University of Rochester, School o Medicine, 601 Elmwood Avenue, Bo 642, Rochester, New York 1464 Affirmative action/equal opportunit employer. 9113(A)

POSTDOCTORAL POSITION

Three-year position available immediately for research on the control of insect reproductive development. Recent Ph.D. (biochemistry, developmental biology, or insect physiology) with experience in nucleic acid chemistry preferred.

Send curriculum vitae and references to:

Dr George M. Happ,
Department of Zoology,
University of Vermont,
Burlington, VT 05405.

An Affirmative Action Employer.
9136(A)

UNIVERSITY OF EXETER

DEPARTMENT OF BIOLOGICAL SCIENCES

RESEARCH ASSISTANT

Applications are invited for a graduate research assistant, to be based in either Exeter or Bath, to work with Drs Todd and Rayner on an S R C.-funded project "Intraspecific Antagonism in Natural Populations of Wood-Decaying Fungi". The genetics, biochemistry and structural changes associated with this phenomenon and its biological and pathological implications will be studied. Preference will be given to applicants with experience in any of these areas. The successful applicant may be permitted to register for a higher degree.

The appointment is for one year in the first instance, starting as soon as possible, with the possibility of renewal for a further two years. The commencing salary will be £3,384 p.a. (£3,634 for the second year and £3,883 for the third year).

Applications, together with a curriculum vitae, giving details of age, qualifications and experience, and the names of two referees should be sent by February 1, 1979, to Dr N. K Todd, Washington Singer Laboratories, University of Exeter, Exeter EX4 4PS
9049((A)

FOOD ENGINEERING

Applications are invited for a

PROFESSORIAL POSITION

in the Department of Chemical Engineering and Applied Chemistry, University of Toronto. The opening relates directly to an expansion of teaching and research in the field of food engineering. The expansion is possible as a result of a Development Grant from the Connaught Committee, University of Toronto. The appointment is in the tenure stream, commencing July 1, 1979. Applicants should possess a Ph.D or its equivalent in chemistry or chemical engineering, and have a demonstrated or potential ability to teach at the postgraduate level and to initiate research. It is expected that the appointment will be made in either the Assistant or Associate Professor rank.

Applications with curriculum vitae and the names of three referees should be sent to:

Professor M. E. Charles,
Chairman, Department of Chemical Engineering and Applied Chemistry
University of Toronto
Toronto, Ontario
Canada M5S 1A4.
9052(A)

PROFESSOR OF PHYSIOLOGICAL OPTICS

The College of Optometry at The Ohio State University with its graduate programme in physiological optics, has an opening at the full professor level for a vision scientist of outstanding calibre. The candidate must be an effective teacher and an active and recognised researcher. A Ph.D (or equivalent) degree is required. Selection and salary depend on ongoing productivity, relevance to this programme's needs, and potential for continuing development.

Send curriculum vitae, bibliography, and three references to: Chairman, Search Committee, The Ohio State University, College of Optometry, 338 West 10th Avenue, Columbus, Ohio 43210, (614) 422-2647 (The Ohio State University is an affirmative action equal opportunity employer.)
9111(A)

GUY'S HOSPITAL MEDICAL SCHOOL
(University of London)

POSTDOCTORAL RESEARCH ASSISTANT

required to join a research group under Dr L. Mallucci in the Department of Microbiology investigating the role of cell surface membrane components in cell growth regulation and cell differentiation. Position available to December 1979 with the possibility of further renewal of grant.

Salary in range £3,910 to £5,506 plus £450 London Allowance and superannuation.

For further details telephone Dr Mallucci.

Applications to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT.
9055(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

FACULTY OF MEDICINE

ASSISTANT PROFESSOR OF IMMUNOLOGY

Immunologist/Immunogeneticist required at Assistant Professor (and Postdoctoral fellow) level to join others in a medical school immunology group. Special research strengths of this group are a good HLA lab (International workshop standard), complement allotyping, collaborative relationships with the blood bank, biochemical genetics, cytogenetics and with clinical investigators, plus the availability of large families. Our group is also involved in teaching, in a clinical immunology diagnostic laboratory and with a transplantation programme.

For more information please write, preferably sending detailed curriculum vitae plus any relevant reprints, to Dr W. H Marshall, Professor of Immunology, Faculty of Medicine, Memorial University of Newfoundland, Canada, A1B 3V6.
9137(A)

UNIVERSITY OF MELBOURNE

Australia

LECTURESHIP

(Limited Tenure)

in the School of Physics

Applications are invited for a Lectureship (Limited Tenure) in the School of Physics. The current research interests of the School are high energy physics, diffraction physics, low energy nuclear physics and theoretical physics.

The successful applicant will be required to teach at an undergraduate level by lecturing or supervising laboratory classes, to supervise research students and to conduct research in one of the above-mentioned areas of physics.

Qualifications: Ph.D. with postdoctoral experience.

Salary: $A15,179 to $A19,940 per annum.

The position may be taken up from September 1979.

Further information, including details of application procedure and conditions of appointment, is available from the Registrar, University of Melbourne, Parkville, Victoria, 3052, Australia, or the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications close on February 28, 1979.
9124(A)

RESEARCH GRADUATE

to undertake studies in relation to human myeloma cultures and their influences on antibody formation etc. Prior experience of tissue culture, lymphocyte culture or bone marrow work is preferred. Excellent opportunity for a Ph D thesis. Salary Whitley Scale A according to experience. Applications, with two referees, to Professor J. R Hobbs, Westminster Hospital, Department of Chemical Pathology, 17 Page Street, London SW1P 2AR, by January 28, 1979.
9110(A)

ROYAL OBSERVATORY, EDINBURGH

SENIOR APPOINTMENT

There is a vacancy for someone with a proven record of success in the management of projects with a considerable technological content to join the Observatory's senior management team.

The Observatory is responsible for three national facilities which support University research: the Image and Data Processing Unit which includes the advanced measuring machine COSMOS; the 3.8 m aperture Infrared Telescope currently being erected in Hawaii, and the UK 1.2 m aperture Schmidt Telescope in Australia. The last two also have associated laboratories at Edinburgh and all three, together with the in-house research, are supported by Technology and Administration Groups. All major activities in the Observatory are managed by senior research scientists who are expected to spend on average half of their time on research. Management duties sometimes involve overseas postings, currently to Australia or Hawaii, for periods of about three years.

The successful candidate for this post will in the first instance be made responsible for overall management of the Technology Group.

Applicants should detail their project management and research experience which should preferably be in the fields of astronomy or instrument development and/or precision engineering.

Salary, depending on qualifications and experience, on the scale £10,043—£11,300 per annum.

SRC

Letters of application, including a full curriculum vitae, should be sent to Mr B McInnes, Royal Observatory, Blackford Hill, Edinburgh (Tel No 031-667 3321) by 31st January 1979.
9056(A)

ORPINGTON HOSPITAL SECTOR

DOMESTIC SUPERVISOR

male or female required for the above Sector, which includes responsibility for the Angus Home, Cudham, a residential home for 28 female adult mentally handicapped residents. The successful applicants will join our team of supervisors responsible for the Domestic Staff in the Sector.

Previous experience preferable. 40-hour week working from 7.30 a m to 4 p.m. Monday to Friday. Weekly earnings £53.54.

Application form from:
Sector Administrator,
Orpington Hospital,
Orpington, Kent, BR6 9JU.
(Tel. Orpington 27050, ext 86.)
9141(A)

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

RESEARCH ASSISTANT

(Grade 1A—
Salary £3,383 to £6,555)

Applications are invited for a Research Assistant, supported by the Cancer Research Campaign, to assist with research into the biochemical mechanisms of virus growth. Experience with mammalian tissue culture would be an advantage. Applicants should possess a degree or equivalent qualification.

Applications, in writing, together with the names and addresses of two referees, to the Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE.
8960(A)

ST HELIER HOSPITAL

Wrythe Lane
Carshalton, Surrey

RESEARCH ASSISTANT

with working experience in immunology in Dermatology Department.

An original enthusiast needed who will help to investigate several aspects of Dermatology including malignancy.

Laboratory staff will consist of two full-time Biochemists, Senior Registrar and Consultant.

Salary according to experience. Post reviewed annually.

Applications to Dr E. Rhodes at above address. Closing date: February 9, 1979
9096(A)

THE BREWING RESEARCH FOUNDATION

require a

MOLECULAR BIOLOGIST/ YEAST GENETICIST

Applications are invited from persons interested in developing recombinant DNA techniques for the genetic improvement of commercially important strains of yeast. Candidates should have (or expect to obtain shortly) a Ph.D in an appropriate area of biochemistry, molecular biology or genetics. Experience in the use of restriction enzyme/DNA cloning methodology would be preferred. Appointment will be to the scientific staff of the Foundation at an appropriate point on the Scientist scale (£4,486 to £6,200). Applications, including curriculum vitae and names of two referees should be sent to: The Administrative Manager, Brewing Research Foundation, Lyttel Hall, Nutfield, REDHILL, Surrey RH1 4HY.
9108(A)

Pharmacokineticist

May & Baker Ltd., is an international Company, manufacturing and marketing a wide range of research based chemical products.

Following a functional reorganisation, we have a vacancy in the Department of Drug Metabolism at our Research Institute at Dagenham, Essex, for a graduate with pharmacokinetic experience.

The successful applicant will lead a small team working on the design, implementation and competent reporting of pharmacokinetic and bioavailability studies. A good knowledge of analytical apparatus and its application is essential and a general knowledge of drug metabolism is desirable.

Benefits of employment include flexible working hours, bonus scheme and contributory pension scheme. Publication of appropriate work is encouraged and the Department has active links with a number of University and Clinical Pharmacokinetic Departments throughout the world.

Please telephone 01-592 3060 extension 2506 for an application form or write with full details of age, experience, qualifications and present salary to Mrs. Phyllis Clark, Personnel Officer (Scientific Staff),

M&B May & Baker

May & Baker Ltd.,
Dagenham, Essex RM10 7XS
quoting reference number 164/N/2. 9097(A)

Re-advertisement

Director

PHLS Centre for Applied Microbiology & Research, Porton Down

The PHLS Centre for Applied Microbiology and Research will take over and develop the civil and health oriented aspects of the work of the Ministry of Defence Microbiological Research Establishment at Porton Down, Wiltshire, on April 1, 1979 Within the Centre there will be reference laboratories for 'special' pathogens and laboratory safety, research laboratories concerned with microbial pathogenicity and vaccines, and laboratories involved in work on microbial products used in therapy, diagnosis and research.

The PHLS Board invites applications for the post of **Director of the Centre.** Candidates (male/female) should have an established research reputation in microbiology or some related aspect of public health, and experience in the management of a laboratory or similar department. While preference will be given to candidates with a medical qualification, applications from non-medical graduates with specially relevant interests or experience will be welcomed.

The duties of the post will include the promotion and co-ordination of the research activities of the laboratories within the Centre, and the integration of these activities with those of the PHLS and the health services generally

The appointment will be under PHLS (NHS) terms and conditions of service Further details of the post and salary scale may be obtained from **The Secretary, Public Health Laboratory Service Board, 61 Colindale Avenue, London NW9 5EQ,** to whom applications, stating date of birth, qualifications, experience, published work, and naming three referees, should be sent by February 12, 1979 The Director of the PHLS, Sir Robert Williams, will be pleased to discuss the post and to make arrangements for those interested in the post to visit the laboratories at Porton 9128(A)

PHLS Public Health Laboratory Service Board.

ROYAL HOLLOWAY COLLEGE
University of London
DEPARTMENT OF ZOOLOGY

N.E.R.C. RESEARCH ASSISTANT

required to work for two years with Dr J W. Lewis on the epidemiology of parasitic infections in small mammals. Post suitable for graduate in zoology/biology with a background in parasitology/mammalogy.

Salary on scale £3,410 to £3,659 plus London Allowance of £450. Starting date to be arranged.

Apply in writing, with curriculum vitae, to the Personnel Officer, Royal Holloway College, Egham Hill, Egham, Surrey. 9109(A)

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

RESEARCH TECHNICIAN

Applications are invited for the post of Research Technician in the Metabolic and Endocrine Unit. The post includes work on problems of hepatic and acid base metabolism and also general laboratory duties. The appointment is for a maximum of three years and is supported by the Medical Research Council.

Initial salary £3,261 to £3,582, according to experience, plus £354 London Weighting.

Further details may be obtained from Professor R D. Cohen, Metabolic and Endocrine Unit, Tel: 01-247 5454, ext 412. Applications, together with the names of two referees, should be sent to the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, within 14 days, quoting reference RTMEU/1/79 9133(A)

THE KENNEDY INSTITUTE OF RHEUMATOLOGY

A postdoctoral or postgraduate

RESEARCH ASSISTANT

is required in the Division of Biochemistry to be involved in projects using culture techniques to investigate the cellular mechanisms involved in the control, development and repair of connective tissue Candidates should have a keen aptitude for research and previous experience of cell culture techniques. The appointment is funded by the Medical Research Council until November 1980

Salary £3,500 to £4,500 depending on age and qualifications.

Applications in writing giving full particulars of experience and qualifications and two referees to The General Secretary, The Mathilda and Terence Kennedy Institute of Rheumatology, Bute Gardens, Hammersmith W6 7DW. 9064(A)

AYRSHIRE AND ARRAN HEALTH BOARD, North Ayrshire and Arran District **SENIOR CYTOGENETICIST** (Science Graduate) required for Ayrshire Area Laboratory to work in a developing diagnostic service for the detection of human chromosome aberrations. Appropriate qualifications and experience in cytogenetics and cell culture are essential. The post will initially be based at Ballochmyle Hospital but will move to the new North Ayrshire District General Hospital in 1979 Further details may be obtained from the Pathology Department, Ballochmyle Hospital (Catrine 182, ext. 23). Applications with full details of qualifications and experience and names of two referees, to the North Ayrshire District Medical Officer, 1 Hill Street, Kilmarnock. 9135(A)

PLANT PHYSIOLOGIST/ BIOCHEMIST

The Agricultural Science Service of ADAS provides farmers and growers with a wide range of vital technical information and advice which is supported by investigational and development work carried out from regional centres across the country.

The officer appointed to this Wolverhampton based post will provide specialist advice to colleagues on physiological and biochemical problems in crop production, and to this end will maintain a close liaison with research workers in Institutes, Universities and Commercial Organisations In addition, the plant physiologist will initiate investigational work, develop and evaluate physiological techniques for use both in the field and the laboratory, and take responsibility for the day-to-day management of some laboratory and field staff.

Candidates (normally aged under 35) must have a Degree or equivalent in plant physiology or biochemistry and at least 3 years' relevant postgraduate experience.

Salary, starting between £4,175 and £5,770, rises to £6,890. There are promotion prospects and a non-contributory pension scheme.

For further details and an application form (to be returned by 2nd February 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). **Please quote ref: S(A)906.**

9104(A)

UNIVERSITY OF STRATHCLYDE

Applications are invited for a post of

RESEARCH ASSISTANT

in the DEPARTMENT OF ARCHITECTURE AND BUILDING SCIENCE to work on heat transfer processes in air-heating solar collectors.

Applicants should be graduates in either physics, mechanical, or environmental, engineering or have a background of practical experience in convective heat transfer work.

Appointment, which is for two years commencing March 1979, would be on Range IB of the national salary scale for research and analogous staff. Commencing salary will be on the scale £3,384 to £3,883 with placing according to qualifications and experience. Superannuation benefit.

Written applications (quoting R2/79) giving the names and addresses of two referees and stating full details of qualifications and experience should be sent to Dr P. P. Yaneske, Department of Architecture and Building Science, Architecture Building, 131 Rottenrow, Glasgow G4 0NG, from whom further information can be obtained. 9082(A)

VOM, NIGERIA
PROJECT DIRECTOR

A project director is required for the newly established Programme on the Biological Control of Tsetse Flies by the Sterile Insect Technique. The position will have overall administrative and technical responsibility, involving the various aspects of the project including production, ecology, and research.

The director should be an entomologist or veterinarian with experience in the Sterile Insect Technique, tsetse control, or ecology or trypanosomiasis.

The preferred date of appointment is April 1979 but a later starting date can be negotiated. Salary will be dependent upon qualifications. Applicants should send curriculum vitae and names of three references to:

Director, Joint FAO/IAEA Division
International Atomic Energy Agency
P.O.B. 590
A-1010 VIENNA, AUSTRIA.

9091(A)

HAREFIELD HOSPITAL
Harefield, Middlesex
DEPARTMENT OF CARDIO-THORACIC SURGERY
Research NUTRITIONIST/ BIOCHEMIST

required for one year.

An interesting vacancy exists for a Research Assistant to work on nutritional/biochemical problems to patients undergoing thoracic surgery. Applicants should preferably be graduates in biochemistry/nutrition. Salary scale: £3,798 to £4,899 plus London Weighting of £354.

Job description and application forms available from Miss P. Durand, Personnel Officer, Harefield 3737, ext. 528. Closing date: 14 days from appearance of advertisement. 9100(A)

QUEEN MARY COLLEGE
University of London
PLANT BIOLOGY AND MICROBIOLOGY DEPARTMENT

Applications are invited for appointment as

GRADUATE RESEARCH ASSISTANT

o work with Dr B. Frankland on an S.R.C.-supported project on phyto-hrome intermediates and induction of germination by irradiation of partially hydrated seeds. Post tenable for up to hree years from April, or as soon as possible thereafter.

Applications from those graduating n summer 1979 will be considered. Initial salary (under review) £3,834 p.a. including London Allowance).

Please apply by letter, giving age, qualifications and names of 2 referees, to The Registrar (N), Queen Mary College, Mile End Road, London E1 NS. 9140(A)

EAST BIRMINGHAM HEALTH DISTRICT
East Birmingham Hospital

A vacancy will exist from January 1, 1979 in the grade of

SENIOR MICROBIOLOGIST

The post is funded by the Medical Research Council as part of a Programme Grant for research into gastro-enteritis viruses, especially rotaviruses. Applicants should have a Ph.D. or equivalent qualification. The research programme is directed: (1) towards propagation of human rotaviruses in tissue culture, (2) determination of rotavirus serotypes and their distribution worldwide. This programme is closely linked with fundamental work on molecular biology of rotaviruses being carried out in the University of Warwick under Dr Malcolm McCrae.

Further particulars may be obtained from Dr T. H. Flewett, Regional Virus Laboratory, East Birmingham Hospital, Birmingham B9 5ST, or telephone Mr J. R. Foster, Senior Chief Medical Laboratory Scientific Officer, on 021-772 4311, Ext. 4086 for further details. 9098(A)

ST. MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Paddington, London W2 1PG
GRADUATE IN PHYSIOLOGY OR PHARMACOLOGY

who will be expected to register for a higher degree, required to join team investigating the mechanism of action to drugs affecting gastric secretion.

Appointment, which can be taken up immediately, is for one year initially with prospect of extension for further two years. Project jointly organised between Departments of Physiology and Academic Surgical Unit.

Further information from Mr S. Knight (Tel: (01)-723 1252, ext. 133, to whom application with curriculum vitae may be sent at above address. 9126(A)

PALEOSERVICES LIMITED OF WATFORD

require a

PALYNOLOGIST

with Mesozoic and/or Paleozoic experience to undertake studies of Well sequences and basin analyses.

Salary nogotiable.

Please apply in writing to: Paleoservices Limited, Unit 2, Polychrome Building, Sandown Road, Watford, Herts WD2 4UB. 9093(A)

ASSISTANT PROFESSOR OF ANALYTICAL CHEMISTRY

—tenure track, fall 1979. Send curriculum vitae with a 250-word outline of your research interests, and request 3 letters of recommendation, to Dr D. H. Freeman (Chairman, Search Committee), Department of Chemistry, University of Maryland, College Park, MD 20742 U.S.A. E.O./A.A.E. 8968(A)

STUDENTSHIPS

UNIVERSITY OF LEICESTER
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a C.A.S.E. research studentship to work on the control of aerobic energy conservation in bacteria during growth in continuous culture. The project will be supervised by Dr C. W. Jones (University of Leicester) and Dr J. Linton (Shell Research Ltd, Sittingbourne). The studentship is tenable from October 1979 and the successful candidate will be expected to read for a higher degree. Applicants should possess, or expect to obtain, a good honours degree in Biochemistry or Microbiology and should write to Dr C. W. Jones, Department of Biochemistry, University of Leicester, Leicester LE1 7RH enclosing a curriculum vitae and the names of two referees. 9101(E)

AGRICULTURAL RESEARCH COUNCIL
Institute of Animal Physiology
Babraham, Cambridge CB2 4AT
RESEARCH STUDENTSHIPS

Applications are invited from recent graduates in Medicine, Veterinary Medicine, or holding a first or upper second class honours degree in Science, and from students expecting to graduate this year, for ARC/MRC Studentships for Training in Research Methods. The following research projects are offered:

1. Effect of stress on glucose metabolism in lactation;
2. Behavioural effects of electrical stimulation of the brain;
3. Nutrition and neuronal development;
4. Protein digestion in ruminants;
5. Protein metabolism in pregnancy and lactation;
6. Hormonal control of gene expression in target tissues;
7. Lymphocyte antigens and histocompatibility studies in sheep.

Successful candidates will be encouraged to apply for admission to Cambridge University as registered research students.

Applications giving birth date, nationality, full details of School and University record and the project favoured, with names of two academic referees should be sent to the Director, Dr B. A. Cross, FRS, by February 28, 1979. 9127(F)

COURSES

THE MEDICAL COLLEGE OF ST. BARTHOLOMEW'S HOSPITAL
(University of London)
RADIATION PHYSICS IN MEDICINE

Application are invited for the

JOINT M.Sc. COURSE IN RADIATION PHYSICS

which extends over one year for full-time students, or two academic years for part-time students. It comprises lectures and practical work on the applications of ionising radiations to Medicine and Health Physics. The Course is open to persons who hold an Honours Degree with Physics as a principal subject, and provides a suitable basis for a career in medical physics. Physicists working with radiations in medicine or industry may be accepted as part-time students. The Course is recognised by the S.R.C. and M.R.C. for the award of Advanced Course Studentships.

Further information and application forms can be obtained from the Secretaries of the Departments of Physics at The Medical College of St. Bartholomew Hospital, Charterhouse Square, London EC1M 6BQ and The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB. 9120(D)

UNIVERSITY OF SOUTHAMPTON
M.Sc./DIPLOMA IN BIOLOGICAL AND CHEMICAL METHODS IN PEST CONTROL

This one-year course, which commences in October, is open to graduates in Biology, Chemistry, Biochemistry, Agriculture or any related subject. It provides instruction in modern techniques and methods used in pest management, and is particularly suitable for graduates in Biology or Chemistry who wish to become proficient in relevant aspects of both subjects.

Further information may be obtained from Dr J. W. S. Bradshaw, Department of Biology, Building 3, The University, Southampton SO9 5NH, please quote reference number 1604/R/N.

FELLOWSHIPS

IMPERIAL CANCER RESEARCH FUND
London, England
VISITING FELLOWSHIPS

The Council of the Fund invite applications for Visiting Fellowship to be held in any of the following the Central Laboratories of the Fun at Lincoln's Inn Fields; Mill Hi Laboratories; the Breast Cancer Unit Guy's Hospital; Cancer Epidemiolog and Clinical Trials Unit, Oxfor University; Medical Oncology Unit St Bartholomew's Hospital; or Tumou Immunology Unit, University College London.

These Fellowships will be open t any applicant, irrespective of citizenship who has (or is about to obtain a Ph.D. or its equivalent and who i temporarily or permanently residen outside the UK. Remuneration wil be comparable with postdoctora fellowships in country of origin; th award will be for one year. Return air fares at the lowest practicable rate will be paid for the Fellow an family, and medical insurance wher necessary. The award constitutes Contract of Service namely to undertake specified cancer studies for stated period. Fellowships are no pensionable.

Application to the Director o Research, Imperial Cancer Researc Fund, Lincoln's Inn Fields, Londo W.C.2, England, should be accompanied by full C.V. and list of publications, the names of two referees an account of the applicant's curren and proposed research extending t about 500 words, and a short accoun of the relevance of the above work t the alleviation of cancer. The laboratory or unit in which the candidat wishes to work should be specified Closing date March 31, 1979. 9020(E)

UNIVERSITY OF KEELE
RESEARCH FELLOWSHIP

Polarography of Organic Cation

Applications are invited from Electrochemists with Ph.D. or equivalen experience for a two-year Researc Fellowship (S.R.C. Postdoctoral Research Assistantship) to work with D P. H. Plesch. Salary in scale £3,883 t £4,631 per annum with membership o Universities Superannuation Scheme Further particulars and applicatio forms from The Registrar, The University, Keele, Staffordshire ST5 5BG England. Closing date, March 3, 1979. 9047(E)

UNIVERSITY OF OTAGO
Dunedin, New Zealand
UNIVERSITY POSTDOCTORAL FELLOWSHIPS

The Council of the University invites applications for University Postdoctoral Fellowships tenable in any Department of the University in 1980.

In addition to contributing to current research programmes, the successful applicants will be required to undertake a limited amount of teaching.

The total value of each Fellowship is NZ\$10,000 comprising both emolument and travelling expenses. The maximum amount paid as emolument is NZ\$8,500. This is taxable in New Zealand. The Fellowship is normally for one year but under exceptional circumstances it may be renewed.

Candidates who are offered a Fellowship must have successfully completed their doctorate before taking up appointment. All candidates must have reasonable expectations of completing their doctorate during the year before that for which the Fellowship is offered.

Further particulars are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University, P.O. Box 56, Dunedin, New Zealand.

Applications close on **March 1, 1979.**

FELLOWSHIPS (continued)

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Further information and application forms are available from Medical Research Council, Training Awards Group, 20 Park Crescent, London W1N 4AL (tel 01-636 5422 ex 240)

CLOSING DATE FOR APPLICATIONS: 28 FEBRUARY 1979.

Late applications cannot be considered.

9080(E)

UNIVERSITE CATHOLIQUE DE LOUVAIN
Belgium

FELLOWSHIP IN PHARMACOLOGY

Applications are invited for a Post-doctoral Research Fellow to investigate the pharmacology of receptor-response coupling processes in cardiac and smooth muscles in the field of digitalis and adrenergic receptors, under the supervision of Professor T GODFRAIND.

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Salary is within the range 30,000 to 35,000 Belgian francs.

Applications with curriculum vitae and naming 3 references should be sent to the

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Place de l'Université, 1
1348 LOUVAIN-LA-NEUVE
Belgium

A further copy should be sent to Professor T. GODFRAIND, Université Catholique de Louvain, Laboratoire de Pharmacodynamie Générale et de Pharmacologie, Avenue E Mounier, 73 UCL 7350, 1200 BRUXELLES, Belgium, from which informations can be obtained. 9063(E)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF PHYSICS

RESEARCH FELLOWSHIP IN THEORETICAL PHYSICS

Applications are invited for a post-doctoral fellowship to study extended solutions, such as solitons and instantons, of non-linear field theories, with a view to applications in statistical physics The fellowship is tenable from October 1, 1979, for up to three years, at an initial salary on a range up to £4,382 per annum (under review).

Further particulars may be obtained from Dr D J Wallace.

Applications should be sent to Mrs E C. P Sears, Staffing Section, The University, Southampton SO9 5NH by March 2, 1979, quoting reference 1605/R/N, giving a brief curriculum vitae and the names of two referees.

9046(E)

ASSISTANTSHIPS

UNIVERSITY OF OXFORD
DEPARTMENT OF THEORETICAL PHYSICS
RESEARCH ASSISTANTSHIPS IN SOLID STATE THEORY

Applications are invited for two Research Assistantships in the Solid State Theory Group. These are funded by the Science Research Council to support an ongoing research programme on the theory of phase transitions and disordered systems, and to give theoretical support to experimental programmes in the Clarendon Laboratory. The appointments are temporary and tenable for one to two years from October 1, 1979. The salary is on the Research Support Scale 1A, currently £3,383 to £6,555 per annum (with U.S.S. membership).

Applications, with the names of two referees, to Professor R. J. Elliott, Department of Theoretical Physics, 1 Keble Road, Oxford, by January 31, 1979. 9084(P)

GRANTS & SCHOLARSHIPS

IMPERIAL CANCER RESEARCH FUND
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A small number of Bursaries will be awarded at the laboratories in Lincoln's Inn Fields and Mill Hill for full-time studies leading to higher degrees in research fields relevant to cancer, that include chemistry, cell and molecular biology.

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Applications are invited from British subjects normally resident in the U.K and not over 25 years of age, who hope to obtain First or Upper Second class Honours Degrees in Science in 1979.

Further details and application forms available from

The Personnel Officer
Imperial Cancer Research Fund
Lincoln's Inn Fields
London WC2A 3PX

Completed application forms should reach the Director of Research at the above address not later than February 14, 1979. 9019(H)

DUBLIN INSTITUTE FOR ADVANCED STUDIES
SCHOOL OF THEORETICAL PHYSICS

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RESEARCH SCHOLARSHIPS

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For further particulars and Forms of Application candidates should apply immediately to **The Registrar, Dublin Institute for Advanced Studies, 10 Burlington Road, Dublin 4, Ireland.**

Completed applications should reach the Registrar not later than March 30, 1979.

9131(H)

CONFERENCES

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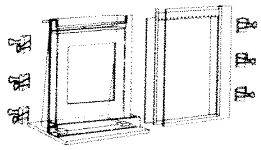

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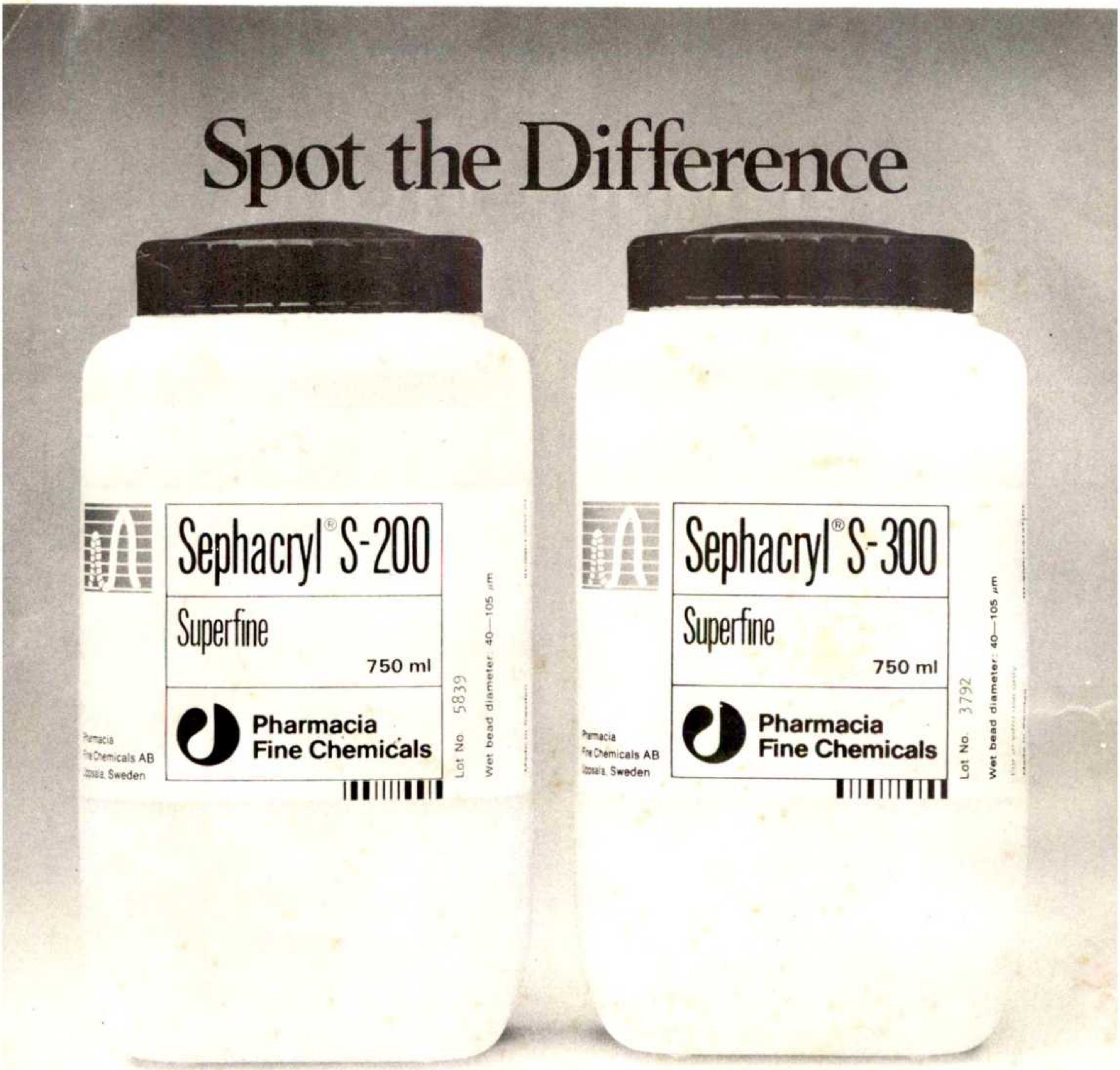

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Cover picture

Scanning electron micrograph of cultured bone marrow cells. The role of interactions between different populations of cells in determining the pathways of haematopoiesis is reviewed on p. 177.

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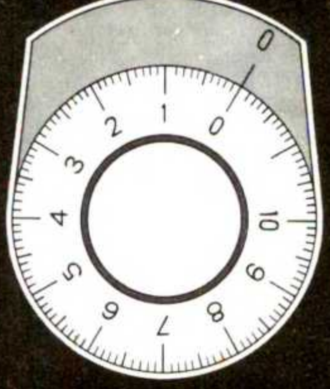

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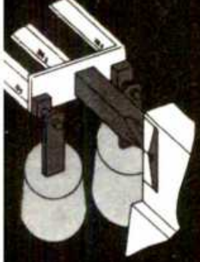

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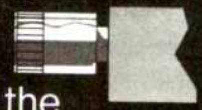

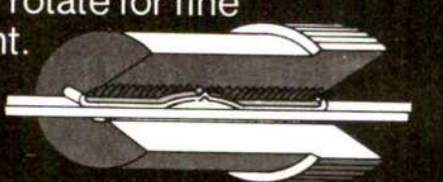

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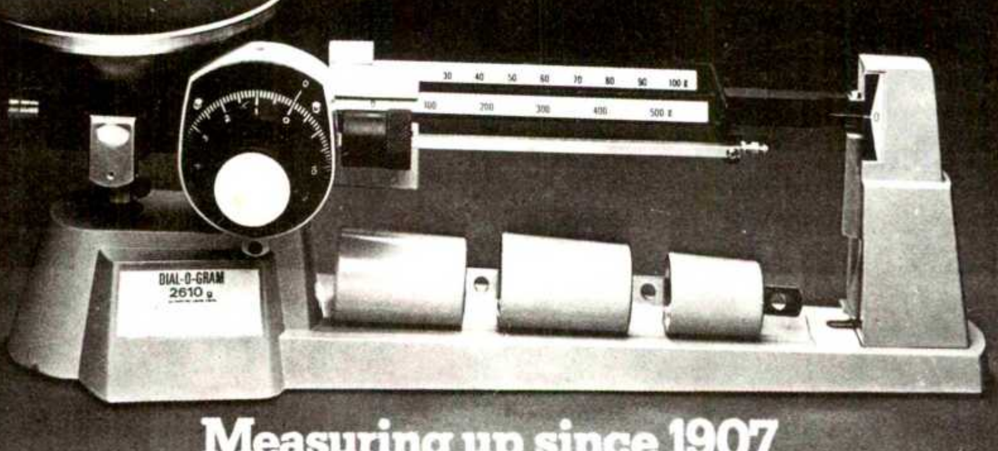

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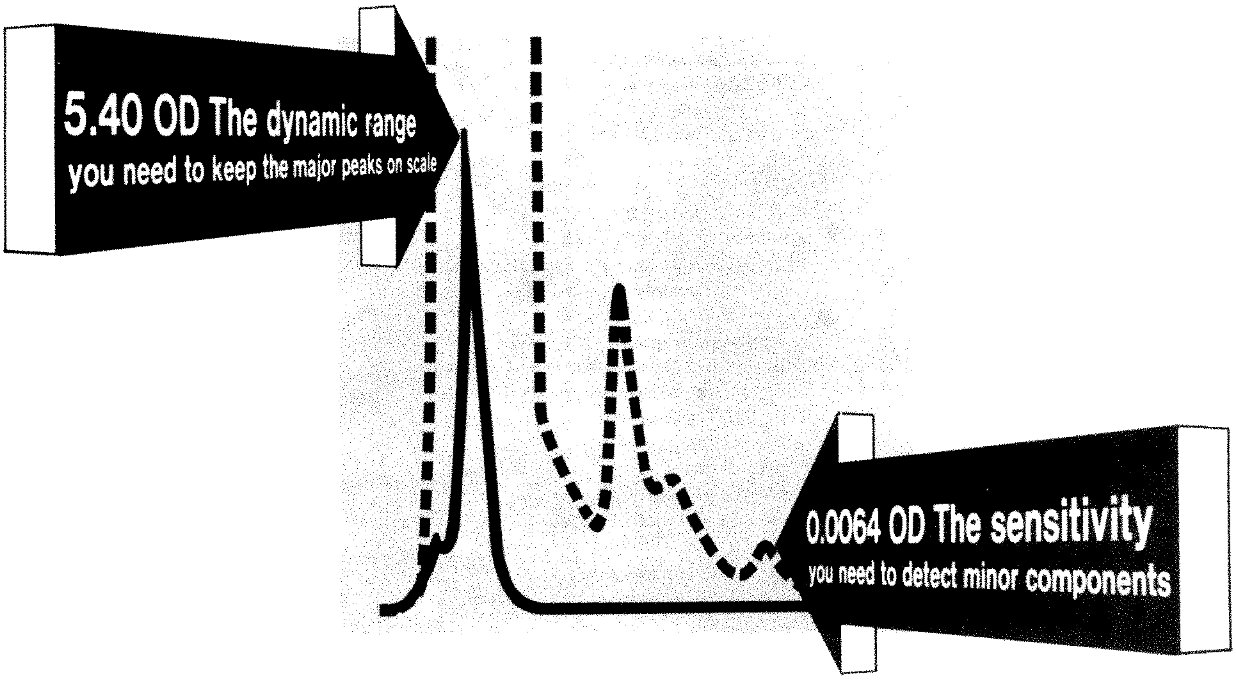

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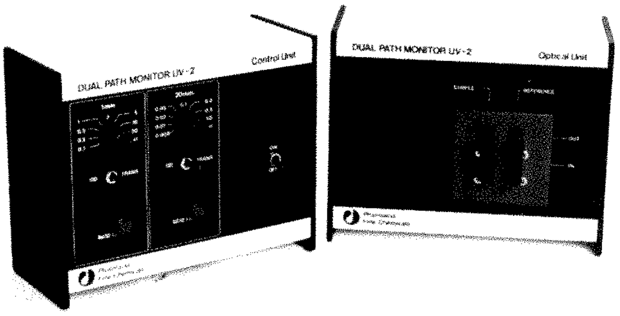

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nature 18 January 1979

The next generation of weapons

In the late 1960s there was widespread debate in the United States on the advisability of installing defences against intercontinental ballistic missiles (ICBMs). At that time the intentions were to install radars around sites to be defended and to fire nuclear-armed rockets at any incoming missile. A defending rocket would not necessarily have to score a direct hit on an attacker—a nuclear explosion in its vicinity would suffice.

Such an anti-ballistic missile, or ABM system would have cost an immense amount and could have hardly guaranteed 100% protection. Further, its very existence would have been an encouragement to the nuclear superpowers to find means of rendering it ineffective. On the other hand, even a partial defence of a major constituent of a nation's deterrent was not to be lightly dismissed. In the event, after long and highly-informed public technical discussions, it became clear that neither superpower was strongly committed to ABM, and a bilateral treaty was signed in 1972 severely restricting deployment.

Military technology has not stood still, however, and new ways of attacking targets such as ICBMs have recently been the subject of a growing amount of interest. Are lasers or particle beam weapons a serious option? Research is being carried out in the United States and almost certainly in the Soviet Union to try and answer this question, and recently debate has moved into the public arena, notably with Richard Garwin's article on beam weapons in the October 1978 issue of the *Bulletin of the Atomic Scientists*, and with a series of articles in *Aviation Week*, also in October, on beam weapons research.

A report has recently been issued by MIT's Program in Science and Technology for International Security (*Particle Beam Weapons*, obtainable from the Department of Physics, MIT). In it, a study group reports in technical terms on the pros and cons of beam weapons in the environments both of space and on the ground.

A beam weapon damages its target by cracking its outer shell, by damaging the electronics or by exploding the warhead's trigger. Typically, to do any or all of these things with a beam of area one square metre, of the order of 100 megajoules would have to be delivered. This is not impossible. On the ground jet engines could be used; in space 60 kilograms of high explosive detonated per second would provide the necessary energy per pulse.

Propagation is more problematical. In space, charged particles are of no use. They leave an opposite charge on the generator which attracts them back towards the gun: it is estimated that a 1GeV, 1,000 amp electron beam with a 1-cm radius would get only a metre beyond the accelerator's exit port. Even if a charged particle beam could propagate, it would be degraded over thousands of kilometres by Coulomb repulsion, and deflected by the geomagnetic field which is imperfectly known in space.

Neutral beams don't suffer the same problems, but even so, gamma-rays and neutrons are not easily concentrated to square-metre cross-sections at thousands of kilometres. Hydrogen atoms look more promising. They can be accelerated in a linear accelerator to several hundred MeV as hydrogen with a second electron in the 2s state, or H^-. The beam can then be aimed with a magnetic lens and the electrons stripped by passage through gas. The divergence introduced by stripping leads to a less than ideally concentrated beam, but even so the components of a hydrogen-beam system appear, in the words of the report 'physically feasible'.

Within the atmosphere, charged beams ionise air and this ionisation makes propagation possible. On the other hand the beam is degraded both in energy and cross-section by scattering. What can happen, however, is that a beam of, say, electrons depositing energy along a path through the atmosphere heats that channel, and within a small fraction of a second air density has dropped to a tenth, or even less of normal. In effect, a hole can be bored through the atmosphere, and for perhaps one-tenth of a second can permit pulses of electrons to travel relatively unimpeded. Many questions remain to be answered, but the possibility of an electron beam of a few thousand amps, consisting of ten thousand or more 100-nsec pulses, being able to damage an incoming missile 'cannot be excluded'. Nor, the group find, is there any compelling reason to conclude that exoatmospheric accelerators for H^- atoms or ground-based electron accelerators could not be built at some time in the future.

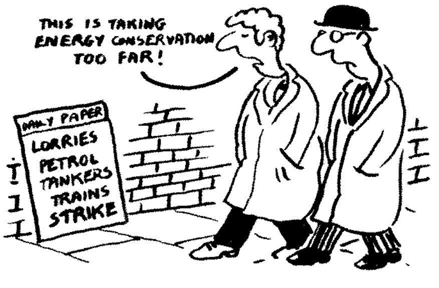
Even if the system can be made to work, however, it has to do so in conditions of war. In space a hydrogen beam might be used either against satellites or to attack ICBMs. At ground level an electron beam might be used as a terminal defence against ICBMs or, at sea, to protect a ship against cruise missiles. In all instances a direct hit is called for (which means accuracies from radars of up to a few parts in a million), and even after such a hit there may be no immediate indication of success. And if the beam misses, it seems near to impossible to determine by how much. Further, the system is open to all the jamming measures that were once discussed in the ABM debate and if the accelerator were in orbit and receiving instructions from the ground that link would be particularly vulnerable. What is more a hydrogen beam would be completely disrupted by the slightest amount of air in space, so a defensive measure could be to fire an explosion in the upper atmosphere, lifting air into space.

The ABM debate is being replayed. An immensely difficult and expensive technical mission, with serious questions at every turn, will be ventured into for fear that the other side just possibly might secure an advantage. There are powerful forces to move the programme forward and spend a lot of money; and for all we know there could be success at the end of the road. There is an arms-control means of stopping development, however. The 1972 treaty noted that if ABM systems 'based on other physical principles' emerged, discussions would be held on limiting such systems. Beam weapons clearly fall into this category. Talks should start soon, even if at present there are no plans for deployment.

Low energy, but lots of loot in UK forecast

Energy needs in the UK in the year 2000 will be at most 3% more than in 1976, and may even be 6% less, according to a report published by the International Institute for the Environment and Development last week. The report emphasises the savings that can be made by introducing proven conservation technologies, and allows for a trebling of Britain's gross domestic product (GDP). In contrast, the present official forecast of the Department of Energy (DOEn) projects an energy growth of some 35% to 70% by 2000 after allowing for a 20% saving through conservation measures.

The IIED forecast may nevertheless not be extreme. The DOEn admitted last week that their 20% conservation figure was a rough one, and said that they had been waiting for nearly a year for a response to it. Their figure was based on consumer research by the Electricity Council and the Gas Boards, advice from the Department of the Environment on the progress of insulation of buildings, "and many other sources", but was open to revision. The IIED report would have its impact on the forthcoming Green Paper on energy futures, said a spokesman, although there was too little time to take it fully into account.



Mr Gerald Leach, the author of the report, said last week that far from having an energy gap the UK had "a great deal of room to manoeuvre on supply options, without extreme measures". Much of the necessary conservation technology — such as heat pumps for domestic space heating and efficient internal combustion engines—will become available in the 1980s, he argued, when the devices will begin to replace existing equipment. Cost savings in running the technology will lead to its adoption by the consumer with little or no encouragement from the givernment, he argues. "We have found that it is astonishingly easy to save energy" said Mr Leach. "We began this work as energy pessimists, but now we are optimists".

Many of the measures are emerging as a result of the 1973 oil crisis, says the report, when it became fashionable as well as expedient to consider ways of saving energy. The effect of this work is mostly yet to come. The Royal Institute of British Architects, for example, has enthusiastically adopted conservation, an attitude which has already resulted in buildings using half as much energy as before for only a 2 to 3% cost penalty, but building stock is replaced only slowly. In the home, lights, cookers, and heaters will soon enjoy "very substantial increases in efficiency". Small-scale heat pumps, such as the one being designed by the Gas Boards, are "at an advanced stage" and will make a dramatic impact. The motor industry is designing cars with a 50% reduction in fuel consumption. Industry uses 40% of primary energy, "but a large proportion of that is in heating poorly insulated buildings, where strict regulation is on the way" says Gerald Foley, one of the team that produced the report. Indeed the government has already adopted measures which will improve industrial conservation. "The result is that all across the board there is slack" said Mr Leach. "For example, we predict a need for only 26-30 GW by 2000, whereas the DOEn assumes 83 GW. Quite clearly there is no need for nuclear; we have assumed 4½ to 6½ GW to keep the industry in business. The breeder reactor could be postponed indefinitely." Even renewable sources were not strictly necessary: the report assumes 4-6 million tonnes of coal equivalent produced in 2000 from alternative sources, compared with 10 in the DOEn projection.

The report may, however, have assumed unrealistic substitution rates. The most uncertain element may be the cost of the new technology, and of

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A low energy strategy for the UK (Science Reviews Ltd and IIED) £7.50. Obtainable from IIED, 10 Percy Street, London W1.

Britain's energy optimist

Gerald Leach, author of *A low energy strategy for the UK* (see above), may see himself as an energy optimist—but the *Financial Times* last week saw it differently. Thinking no doubt of the markets lost to the energy supply industry, the subeditor had headlined the story about Leach's report "Bleak forecast for UK energy". But Gerald Leach's interests do not lie with big business.

Gerald Leach is 44, and left science writing and broadcasting (for the BBC and the *Observer*) in 1972. At the *Observer* he had become interested in the works of Commoner and Ehrlich, and had accumulated "shelves of fascinating books with no time to read them. As a journalist I felt I was skating over an ocean." Then he met a representative of the Ford Foundation at the Stockholm environment conference, cheque book in hand, and his new career began.

He produced a series of reports for various bodies on energy matters, including *Energy and Food Production* (for IIED), *Fuel conservation: options for aviation* (for OECD), *Nuclear energy balances in a world with ceilings* (for IIED), and gave evidence on energy futures to the Windscale inquiry on reprocessing. He defended "Limits to growth" at the Royal Society, did "a big splash" in the *Observer;* but "I began to feel uncomfortable. Early on he realised the importance of "saturation" effects—such as a ceiling on the number of cars, or the temperature of a house, or conservation. Growth might limit itself.

"Early in 1976 I'd finished a project and I went down to Her Majesty's Stationery Office to read all the government reports on energy. There turned out to be only 4 pages on futures." But the US was much further ahead. "My first task was to convince the Ford Foundation that we were so far behind". Then he got the grant, and the study was under way under the wing of the International Institute for the Environment and Development. "The original proposal to Ford was on alternative sources with a bit of conservation" said Leach. "Then we began to feel strongly about disaggregation", calculating energy futures by adding up all possible uses rather than using macroeconomic trends. It also emerged that previous reports were unduly moderate on conservation. The result was the low energy prediction just published. □

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fuels which will affect substitution. "We will be attacked by economists" admits Leach. The report has adopted apparatus replacement rates of some 5% per year after the new technology becomes available. Thus heat pumps have a 15-20% penetration of the market by 2000. But in housing the report allows until 2010 before all houses have 4 inches of loft insulation, and cavity walls are filled.

So far, the Department of Energy response has been friendly, and mildly defensive. "My feeling is that there is a lot of work to do yet in conservation in our methodology" said a spokesman. "We are only scratching the surface . . . But has Gerald Leach only taken the most favourable answers from all his inquiries? In our last green paper we took advice from the department of transport, the oil companies, and car manufacturers among others. Most thought that 20% conservation was ambitious. Mr Leach is applying current best practice across the board, and concludes that one can increase that 2 or 3 times. That may be wishful thinking."

The team responsible for the IIED report consisted of Gerald Leach, Christopher Lewis, Frederick Romig, Ariane van Buren, and Gerald Foley.

Robert Walgate

. . . and the view from across the Atlantic

Forecasts of US primary energy demand in 2000 AD

(in quads = 10^{15} BTU)
(1975 consumption 75 q)

Year of forecast	Source of forecast			
	beyond the pale	heresy	conventional wisdom	superstition
1972	125 *Lovins*	140 *Sierra Club*	160 *AEC*	190 *Fed Power Comm'n*
1974	100 *FF (Zero growth)*	124 *FF (Tech. fix)*	140 *ERDA*	160 *Edison Electric Inst.*
1976	75 *Lovins*	89-95 *v H and W—Lovins*	124 *ERDA*	140 *Edison Electric Inst.*
1978	33 *Steinhart (for 2050)*	63-77 *CONAES panel (for 2010)*	95-100 *CONAES, DOE-IEA*	123-124 *RDE—Ralph Lapp*

Energy forecasts have been falling in many countries, not only the UK. Amory Lovins has communicated this table of US forecasts for the year 2000, produced by various bodies over the last six years. "Read diagonally, the matrix has considerable predictive power" says Lovins. The September 1978 Department of Energy forecasts were not known when the table was first prepared. However, there is considerable subjective judgement involved in the choice of columns and of forecasts to fit them. Read literally, the matrix predicts that the conventional 1980 US forecast for the year 2000 will be some 70 quads, less than 1975 consumption.

Abbreviations used: AEC, Atomic Energy Commission; FF, Ford Foundation; ERDA, Energy Research and Development Administration; vH and W, von Hippel and Williams; CONAES, the Committee on Nuclear and Alternative Energy Systems of the US National Research Council; and DOE, Department of Energy. In 1976 Lovins' two forecasts were private (75q) and public (95q, published in *Foreign Affairs*). In 1978 the CONAES panel used three scenarios, as did the DOE (varying with projected oil price). ☐

Black year for Soviet refusniks

1978 was, in general, a black year for Soviet refusnik scientists, marked by increasing harassment and the imprisonment or exile of a number of the best-known of them. However, the year ended on a note of partial triumph—in the last week of December the "Sunday seminar" for refusniks achieved its long-standing aim of an international meeting with the participation of scientists from USA, France and the UK.

Inevitably a number of the intending foreign visitors failed to receive the necessary visas. Five of the eight would-be participants from the US had their visas revoked—evoking a statement of censure from the Committee of Concerned Scientists saying that it "found it unconscionable for the USSR to seek to prevent American scientists from engaging in scientific discussions with Soviet colleagues". The conference, it claimed, was "an excellent example of the kind of unofficial, non-governmental exchanges provided for by the Helsinki Final Act."

"Non-governmental" exchanges do not fit easily into the Soviet concept of science, and previous attempts of the seminar group to hold an international meeting were less successful. In 1974, no foreign visas were issued and the leading Soviet participants were arrested. Nevertheless, the proceedings of the "Conference that never was" were published in the regular manner, and limited foreign participation was permitted in the April 1977 meeting to commemorate the fifth anniversary of the founding of the seminar.

The apparent acquiescense of the Soviet authorities with regard to last month's meeting seem due rather to international pressure than to any change of heart. Continuing routine surveillance of the venue—the flat of Viktor Brailovskii—during the seminar, and a thorough police search with confiscation of his recent scientific material and his Lenin library ticket made this clear. A number of other intending participants suffered like harassments.

Nevertheless, the meeting itself took place. The main purpose of last months gathering was to bring the refusniks abreast of recent Western developments.

The chosen title "collective phenomena" allowed a wide range of subjects. The US participants presented work in statistical physics and vortex motion in superfluid ^{4}He, while the French contributions ranged over crystal physics, axiomatic quantum field theory, one-dimensional conductors, and what one participant described as a "very pretty" result in percolation theory. Since this last contribution required no specialist background, it was of particular value to the refusniks, many of whom have inevitably fallen irrevocably behind in their original field, and who therefore wish to extend their acquaintance with other subjects.

Local contributions were, understandably, somewhat hampered by the extremely restrictive nature of refusnik life—lack of access to experimental facilities and to new results in particular. Nevertheless, a wide range of subjects was attempted, including solitons, plasma physics, and quantum field theory. Yurii Orlov, one of the few non-Jewish members of the original seminar group, could not be present in person—he is at present serving a 12-year sentence for "anti-Soviet agitation and propaganda". A paper of his on "Wave logic", an alternative to conventional two-valued logic, more appropriate to quantum mechanics was presented in absentia.

Another gentile who has always taken a keen interest in the Seminar, is academician Andrei Sakharov. To the delight of all participants, Sakharov attended the first session. Other notable Soviet participants included Aleksandr Lerner, Grigorii Rozenshtein, Yurii Fishman, Yurii Gol'fand and Yakov Al'pert.

Vera Rich

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Psychosurgery supporters sued for malpractice

David Dickson reports from Boston on a little-publicised court case that has revived debate on the psychiatric use of surgery

Two prominent supporters of the use of surgery to control the behaviour of individuals are being sued for malpractice by a former patient who, it is claimed, is now confined to a mental hospital as a direct result of the treatment that he received.

The charge of negligent treatment has been made against Dr Vernon H. Mark, Director of the Neurosurgical Service at Boston City Hospital, and Associate Professor of Surgery at Harvard Medical School, and Dr Frank R. Ervin, now Professor of Psychiatry at the University of California in Los Angeles.

Dr Mark and Dr Ervin became the centre of a fierce public controversy in 1967 when, in a letter to the *Journal of the American Medical Association*, written with Dr William H. Sweet, Professor of Surgery at Harvard Medical School, they suggested that brain dysfunction in individuals might have contributed to the urban riots that swept several American cities in the late 1960s—and that people with 'low violence thresholds' should therefore be diagnosed and treated before they contributed to further such tragedies.

Three years later, Drs Mark and Ervin published a book *Violence and the Brain* in which they wrote that they intended to stimulate a "new biologically-oriented approach to the problem of human violence" and that this should lead to a "more rational approach" to the problems of violence.

The suit claiming malpractice has been filed by the mother of Mr Leonard A. Kille, previously a research and development engineer with the Polaroid Corporation. It is being handled by the Washington law firm on which Health Secretary, Mr Joseph Califano, was once a senior partner.

While working for Polaroid, Mr Kille headed a team that was responsible for a number of patents on the land camera. He received treatment, including brain surgery, from the two defendants in 1966 and 1967 and he is generally taken to be the patient 'Thomas R.' described in *Violence and the Brain* as a "brilliant 34-year-old engineer with several patents to his credit", whose treatment Dr Mark and Dr Ervin say represented the first time they were able to demonstrate that systems in the limbic brain both start and stop attack behaviour.

According to evidence presented to a jury which is currently considering the charges in a Boston court-room, Mr Kille was referred to the Boston General Hospital in 1966 after a history of epileptic seizures and outbursts of violent behaviour mainly directed towards his wife. He was diagnosed as having psychomotor seizures and 'personality pattern disturbance', and subsequently as suffering from temporal lobe epilepsy.

Following apparently unsuccessful attempts by the hospital to treat him with a variety of drugs, Dr Mark and Dr Ervin implanted a series of electrodes in his brain, the stimulation of certain of which relieved his aggressive symptoms. They subsequently performed an operation by making small bilateral lesions in his medial amygdala through heating the electrodes.

Immediately after the operation, according to evidence presented in the court, Mr Kille's behaviour seemed to have improved. At one point for example, he said that he 'felt like a new person'. However, shortly after his release from hospital his violent tendencies returned, and a month later he was admitted to a west coast hospital where he was described as "hallucinated, delusional and confused" and diagnosed as schizophernic and paranoid.

A succession of hospitalisations and subsequent discharges followed, with continued signs of aggressiveness and belligerence. Mr Kille was eventually placed under permanent care at the Bedford Veterans Administration hospital, 25 miles north of Boston, where he is now confined. In the suit that has been filed on his behalf, it is now claimed that he was "rendered of unsound and unbalanced mind" as a direct result of negligent treatment by Dr Mark and Dr Ervin.

In particular, it is alleged that the surgeons failed to inform him fully of the risks and ramifications of the surgical procedures that they were recommending, and that they therefore failed to obtain his informed consent. A charge of battery resulting in permanent injury and incapacitation has been made and damages of $2 million are being sought.

During the court proceedings, the plaintiffs have claimed that the surgeons considered that they were treating a behavioural, rather than a medical, condition (in their book they describe T. R.'s chief problem as being his violent rage), and that although the procedures they were adopting should have been described to Mr Kille and his wife as experimental this was not in fact done.

Suggesting that there might also have been a conflict of interest between medical and scientific priorities, the plaintiffs have pointed out that Dr Mark told a neuroscience seminar in December 1966 that inserting electrodes in the brain for therapeutic reasons served a dual purpose since it had "real value both to the patient and to our scientific enquiry".

They have also quoted a passage from a later printing of *Violence and the Brain* in which Dr Mark and Dr Ervin state that "four years have passed since the operation, during which Thomas has not had a single episode of rage. He continued, however, to have an occasional epileptic seizure with periods of confusion and disordered thinking". This indicates that the surgeons were primarily concerned with altering their patient's behaviour rather than his medical condition—and that they felt they had successfully treated the former.

The defendants have replied to the various charges by saying that Mr Kille was diagnosed as suffering from temporal lobe epilepsy, which gave rise to his seizures, and that given the state of medical knowledge at the time, as well as the patient's failure to respond to more conventional treatment, the surgical procedures that they took were appropriate.

They also claim that during the meeting at which Mr Kille and his wife formally gave their consent to the amygdalectomy operation, it was explained that there was roughly a one-third chance that the operation would successfully relieve his symptoms, a one-third chance that there would be some improvement, and a one-third chance that there would be no improvement or a deterioration.

Finally responding to the published description of the patient T. R., Dr Mark told the jury that this was a composite case history with detail taken from a number of patients, and that it should not be taken as describing exactly the case of Mr Kille.

The whole case has revived the debate on the ethics of psychosurgery which flourished at the beginning of the decade. Two years ago, following widespread public concern on the issue, the National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research issued a report which, to the surprise and disappointment of some and the delight of others, gave psychosurgery its approval in some circumstances.

Though professing to an initial scepticism, the report drew on studies showing that a large number of

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patients had been successfully relieved of pain or depressive symptoms as a result of such surgery to support the contention that it was a potentially beneficial therapy.

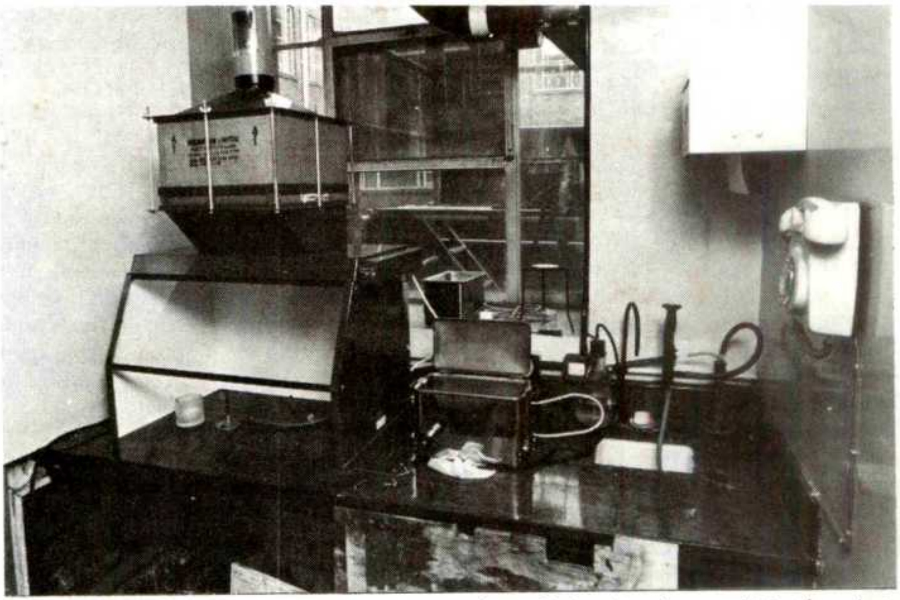
Others still claim, however, that although psychosurgery may be effective in alleviating symptoms such as pain and depression, this is frequently at the expense of sacrificing other aspects of the individual's identity, such as emotional responsiveness and creativity. It is also claimed that the psychosurgeons approach reduces social behaviour to a purely biological phenomenon, thus denying a range of environmental factors which alternative forms of treatment, such as psychotherapy, can take into account.

"There is no scientific foundation for attempting to control a specific aspect of social behaviour by destroying a portion of the human brain", says Dr Stephan Chorover, Professor of Psychology and Brain Science, at the Massachusetts Institute of Technology, who has given evidence during the court hearings. "The main thrust of this approach to social problem solving is that it tries to localise the problem in 'defective individuals' and therefore embodies a decision not to search for problems in the larger social system".

Whichever way the court decision goes, it is unlikely to resolve the wider philosophical issues surrounding the use of psychosurgery, since the matters on which the jury has been asked to judge relate essentially to the adequacy of the medical procedures followed prior to and during the treatment.

At the same time, however, it could provide a precedent for further legal actions. Already a second case is being brought on behalf of a girl who, at the age of 19 but without her consent, and after a series of electric shock treatment but minimal psychotherapy, had a cingulotomy—a lesion of the cingulum—performed by another Boston surgeon. This case is scheduled to be heard shortly. ☐

More dangerous smallpox labs?



Above: the smallpox laboratory at Birmingham University from which the virus escaped last August. A possible route of infection—through a cable duct—appears bottom left.

AS FAR as the World Health Organisation (WHO) is aware there are now nine laboratories in the world holding live smallpox virus; but only three of them—those in London, Hamburg and Atlanta—are known to match up to WHO smallpox laboratory safety recommendations. The laboratories at St Mary's Hospital, London and the Centre for Disease Control, Atlanta are to become WHO collaborating centres for poxvirus research; so is the Research Institute of Viral Preparations, Moscow, which is currently making modifications to come up to the WHO standard.

The Institut fur Schiffs und Tropenkrankheiten in Hamburg, the Rijks Institut voor de Volksgezondheid, Bilthoven, the Netherlands, the US Army Medical Research Institute for Infectious Diseases and the American Type Culture Collection are all considering either destroying or transferring their viruses to one of the WHO collaborating centres. The latter three laboratories have not been visited by WHO. The other two laboratories currently holding smallpox virus are in Peking (which WHO has no plans to visit) and South Africa, which does not come up to standard, is storing the virus, but doing no research. Only the Centre for Disease Control in Atlanta is doing smallpox research at the moment.

The risks of laboratory infection should be seen in proportion, however. The WHO smallpox eradication unit has reduced the death rate from the disease from one million per year 10 years ago to probably zero this year. And smallpox is not an easily transmitted disease: it is not as infectious as flu or measles, for example. ☐

● **Correction:** six white coats per laboratory worker were recommended by the Howie report on a code of practice for the prevention of infection in clinical laboratories, not the Godber report on the handling of dangerous pathogens, as indicated in last week's leader.

Scientist may sue over "Golden Fleece" award

THE Supreme Court has agreed to rule on whether Senator William Proxmire committed libel when he made a "Golden Fleece" award to a scientist engaged in a study for NASA of teeth-clenching by monkeys.

Senator Proxmire, who is chairman of the Senate Appropriations Subcommittee responsible for the budgets of both NASA and the National Science Foundation, has frequently made such awards to esoteric- or naive-sounding research projects, which he claims to be an unncessary drain on the public purse. One recipient of the award was a research scientist, Dr Ronald R. Hutchinson, of Kalamazoo, Michigan, who, according to Mr Proxmire, has received more than $500,000 in federal funding for research that includes measuring the latent aggression of laboratory monkeys under various conditions by recording electrical impulses in the jaw muscles.

Although NASA has said that the work was of particular importance in its efforts to identify aggressive personality traits in selecting crews for long space flights, Mr Proxmire called the research an outrageous example of "wasteful, extravagant, stupid spending".

A lower court rejected a libel action for $8 million brought by the research worker partly on the grounds that he had become a "public figure" by soliciting government grants and publishing occasional articles in professional journals. Senator Proxmire also claimed legislative privilege. The Supreme Court has now agreed to hear an appeal from Dr Hutchinson, who claims that, as a result of the award, he has suffered injury to his reputation, illness, mental anguish and prospective loss of economic advantage. ☐

David Dickson

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news in brief

MRC calls for more research students despite poor job market: In a generally bleak annual report, the UK Medical Research Council (MRC) has announced it is to recruit young scientists "despite the current shortage of longe term career openings in the universities". However, the MRC will introduce three new measures to alleviate some of problems of the most able research workers and to bolster confidence that the UK will provide opportunities for scientists of "really high ability to develop their talents". The new measures are: a salary grant scheme to permit university staff to work full time on research projects of a very high standard; a similar scheme to provide a small number of clinical research posts for clinical academic staff in the country's medical schools; and five-year renewable fellowships for "particularly able people" to bridge the period between a researcher's first employment and subsequent permanent post. The MRC aims to appoint three such fellows a year, building up to a total of 15.

The report makes no mention of scientists currently in employment on short term contracts. According to a recent AUT estimate there are 5,000 researchers in the UK on short-term contracts funded by the MRC and an additional 1,700 registered as unemployed with the Department of Employment. (*Medical Research Council Annual Report* 1977-78 (HMSO) £3.75).

Czech Chartist jailed for nine months: Dr Jaroslav Sabata, a former professor of psychology and one of the three spokesmen of the Czechoslovak Charter 77 human rights group, was sentenced last week to nine months' imprisonment on the formal charge of "insulting a police official". Dr Sabata, who was a prominent Communist Party official during the "Prague Spring" of 1968 and who has since served five years of a seven year prison sentence for dissident activities, is a fervent advocate of closer links between the human rights movement in East European countries. His arrest last October near the Polish-Czech frontier took place during a series of meetings between the Czech Chartists and the Polish "Committee for Social Self-Defence". Authorities on both sides of the frontier tried to prevent the meetings.

During the trial, the procurator described Sabata as a "criminal" whose activities threatened the whole Czechoslovak people—a clear indication that his real offence in official eyes is his constant campaigning for personal, political and academic freedom.

Australia restructures its science planning: Following the report of the first inquiry into the functioning of the Commonwealth Science and Industry Research Organisation (CSIRO) since 1949, the Australian Government has announced a restructuring of CSIRO's top management to "facilitate closer liaison between researchers and users of research results, particularly industry". Described as an "update" rather than a "shake-up", the move organises the 37 CSIRO divisions with their staff of 7,000 into five institutes, each with a new director. The old executive structure of five full-time members and four part-timers has been replaced by a new policy-making body composed of three full-time members plus the directors of the new institutes. The identity of the 37 divisions has been preserved and the autonomy of CSIRO is not affected.

The new groupings and their directors are: Institute of Animal Sciences (Dr K. A. Ferguson); Institute of Biological Resources (Mr M. V. Tracey); Institute of Earth Resources (Mr I. E. Newman); Institute of Industrial Technology (Professor Emeritus H. W. Worner); Institute of Physical Sciences (director to be appointed); and the Bureau of Scientific Services (Mr S. Lattimore).

Ustinov on Einstein: Peter Ustinov (right), actor, writer and director, will on 14 March introduce and narrate a special two-hour BBC television programme celebrating the centenary of Einstein's birth. Ustinov, for whom mathematics and science were a total mystery when he was at school, has proved to be "a perfect student", according to producer Martin Freeth. The programme will concentrate on general relativity, the most difficult of Einstein's theories and features experimental and theoretical physicists Kenneth Brecher, Wallace Sargent, Irwin Shapiro, Roger Penrose, John Wheeler, Sidney Drell and Dennis Sciama. Written by Nigel Calder, the programme was filmed on location at the 107 inch reflecting telescope at the McDonald Observatory of the University of Texas. The programme will also use supporting film from other research centres showing current research results that support Einstein's theory.

Expanding Third World does not bring unemployment: Developing countries no longer simply sell raw materials in exchange for manufactured goods but are becoming manufacturing nations in their own right, and their increase in productive capacity is now being felt in the industrialised countries. Between 1972 and 1976, imports of manufactured goods to EEC countries increased from $27 billion to $76 billion. Experts consulted by the EEC have now cautioned against imposing trade barriers, saying that European industry must be adjusted to make it more competitive by "transferring production from stagnant or declining industrial sectors to those with a more promising outlook."

The effects on employment, while potentially serious, are held by EEC experts to be insignificant. Industrial employment began to decline in 1965. Using the UK as an example, the job losses attributable to Third World competition are 0.05% in textiles, 0.4% in the shoe industry, 0.8% in cotton fabrics and 1.7% in clothing. The real problem, say the experts, is the shifts caused by the new international division of labour which is causing job changes for European workers rather than unemployment *per se*. (*euroforum*, 12 December, 1978.)

Miners oppose nuclear power station near Newcastle: The National Union of Miners (NUM) is mounting a campaign to oppose a Central Electricity Generating Board plan to build a nuclear power station at Druridge Bay, near Blyth. Mr Sam Scott, secretary of the Northumberland region of the NUM, said: "The CEGB is proposing a nuclear plant on top of one of the biggest combined coal pits in Europe and parallel to parkland held by the National Trust. It's stupid".

But Mr A. D. Nash, of the CEGB's planning department, said the plan was "only an exploration of options". There was no commitment to nuclear power. CEGB projections based on an annual 2%-3% growth in demand indicated that new capacity would be needed by the end of the 1980s which could be supplied either by coal or nuclear power. With the six-year lead times necessary to build new plant, it was necessary to explore the options now, Mr Nash said. The miners, along with the National Coal Board, are seeking assurances from the CEGB that coal consumption in the area will not be cut.

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Earthscan

Why the world's deserts are still spreading

The fight against desertification is being held up while the politicians wrangle, **writes Anil Agarwal**

THE hopes generated by the United Nations Conference on Desertification (UNCOD) for achieving zero desert growth by the turn of the century, seem to have all but disappeared in just over a year. UNCOD, held in Nairobi in autumn 1977, was widely hailed as one of the most successful UN conferences of the decade. It created an unprecedented awareness about the magnitude of the growing deserts problem, prompting even US President Jimmy Carter to declare that desertification is "one of the most serious problems of our age." It also made a very significant intellectual contribution, unlike most UN conferences, by increasing the understanding about the processes of desertification.

Now, however, there is disillusionment, inaction and fading of interest, according to Professor Mohamed Kassas, Professor of Plant Ecology at the University of Cairo, who played an important role in the organisation of UNCOD. Professor Kassas, who was recently elected President of the International Union of Conservation of Nature and Natural Resources (IUCN), claims that neither the governments of the countries affected by deserts, nor the rich donor countries who were expected to provide substantial funds, have yet shown any serious commitment to combatting desertification.

Most of UNCOD's recommendations were directed towards national governments. It was hoped, for instance, that each country would set up an official focal point to coordinate its desert-related activities. In many developing countries, however, there is often such a gap in communications that one arm of the government is unaware of what the other is doing. While one ministry may be preparing programmes to control deserts, others may be pushing through agriculture and animal husbandry projects that result in increased desertification. The need for coordination is thus important. However, no African government has yet set up such a focal point.

The plans of the United Nations Environment Programme (UNEP) to set up a consultative group for desertification control (DESCON) have also not proceeded very far. UNEP brought together possible donor and recipient countries in May 1978 for discussions. These countries agreed in principle that a mechanism like DESCON is required to mobilise resources for anti-desertification programmes in developing countries. But few of them have yet officially written to UNEP even to confirm their membership of such a group. In these circumstances, the substantial financial assistance that is required to carry out the numerous programmes suggested at UNCOD, is quite unlikely to materialise.

Politics, both national and international, lies at the heart of this lack of interest. At the international level, there is the growing disillusionment of the rich Western countries with the United Nations system, which is reflected in their growing keenness to channel financial assistance directly to recipient countries through bilateral agreements, instead of through the United Nations agencies. This reaction is setting in partly because of the inefficiency and sluggishness of the UN agencies, but even more so because of their involvement with laudable but contentious issues like the New International Economic Order, which irritate many influential politicians in the West. The same rich countries which are fighting shy of making any commitment to anti-desertification programmes through UNEP have pledged over $2,000 million for the development of the Sahel countries through the Club du Sahel, a non-UN organisation.

At the regional level, it is the lack of political will amongst Third World governments to overcome their political differences with each other that bedevils anti-desert programmes. Realising that neither deserts nor desert people, like nomads, recognise political boundaries, UNCOD had tried to formulate solutions to the desert problems with a transnational perspective. UNCOD therefore proposed giant transnational projects like a green belt across North Africa, another green belt across the Sahel, a joint livestock management programme in the Sudano–Sahelian countries, a joint groundwater management programme covering northeast Africa and the Arabian peninsula, and two projects to monitor desert creep in southwest Asia and south America; and it expected feud-ridden countries like Egypt and Libya, Libya and Sudan, Morocco and Algeria, Arab Yemen and People's Yemen, India and Pakistan, and Chile and Argentina, to co-operate in their implementation.

UNEP had indeed taken the bold step to prepare feasibility studies for these transnational projects and get as many governments as possible to sign formal protocol documents for their

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Edge of the Sahara: the bare sand is caused by grazing of livestock and cutting of trees for firewood.

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implementation well in advance of UNCOD. As a result, when the conference was convened, there was considerable optimism about the seriousness with which they would be implemented. Since then, however, there have only been statements of good intention by political leaders. President Houari Boumedienne, for instance, had assured Dr Mostafa Tolba, Executive Director of UNEP that Algeria would press ahead with the North African green belt covering Algeria, Egypt, Libya, Morocco and Tunisia. But there are very few signs of the speed and urgency that is required. These five North African countries together lose a total of about 100,000 hectares of productive land to advancing deserts every year, which such a project could prevent.

Nepal has expressed an interest in joining India, Iran, Pakistan and Afghanistan in the south-west Asian desert-monitoring-project but the political unrest in Iran, where the project's headquarters were to be situated, and the sudden change of government in Afghanistan have delayed this project too.

The politically calm Sahelian region is, however, faring relatively better, with a growing institutional base for desert control. An Interstate Committee for Drought Control in the Sahel (CILSS) has been set up which involves senior Ministers, and it is receiving international assistance through the Club du Sahel. Regional scientific research is being undertaken by the new Institut du Sahel situated in Bamako, Mali. The United Nations Sudano-Sahelian Office based in Ougadougou, Upper Volta, is being expanded to help all the countries in this region to undertake desert control projects such as those proposed at UNCOD.

The Institut du Sahel will hopefully become a model for regional cooperation in deserts-related research and training. Since UNCOD, UNEP has organised two training courses for scientists and officials from developing countries, one on sand dune stabilisation in China and another on soil salinity and improved management of pastures in the USSR. Considering the enormous social and ecological differences between different desert regions of the world, clearly it would be much better to set up regional centres for desertification training and demonstration, and organise such courses within the regions themselves.

But because of the difficulties in organising effective regional cooperation in combating desertification outside the Sahelian region, Professor Kassas believes that stress should now be placed on encouraging national anti-desert programmes. These programmes could be integrated with national land and water management schemes which aim to raise and stabilise the productivity of the arid regions. National planners, who have to make the best of scarce national resources, are more likely to be attracted by rational desert development schemes, rather than those which are seen as simply desert control schemes or desert monitoring exercises.

The problem of growing deserts is indeed urgent. In the early stages of desertification, the cost of reclaiming land is relatively modest, but if neglected, the cost climbs steeply, until reclamation becomes economically impractical. Global land-loss from man-made causes is already estimated to cover an area bigger than Brazil. Estimates presented at UNCOD revealed that more than 680 million people are living on land that can no longer support permanent agriculture, or is showing signs of steady deterioration. These lands today produce a very large proportion of the global production of cereals, fibres and meat. It is therefore vital to restore them back to health.

Desertification issues will be raised at yet another international conference in Rome in July, 1979: the World Conference on Agrarian Reform and Rural Development (WCARRD), being organised by the Food and Agriculture Organisation. The latest WCARRD newsletter states: "There is no technology problem. Answers lie in mobilisation of national and international resources and popular participation in anti-desertification programmes. These issues will be on the WCARRD agenda." It can only be hoped that WCARRD will succeed where UNCOD has failed. ☐

Anil Agarwal is assistant director (editorial) of Earthscan.

African chemists hit back at foreign technology

THE conflict of interest between 'foreign' companies in developing countries and their hosts "can, and does, have a significant adverse effect on the growth of industrialisation in many developing countries and is at least one of the major causes of the slow pace of the development of industries in these countries", according to Professor D. E. U. Ekong, Vice-Chancellor of the University of Port Harcourt, Nigeria. And he is not alone in his criticisms. At a recent UNESCO symposium on university-industry interactions in chemistry, all the African delegates agreed that the system of itinerant expatriate advice is totally irrelevant to African needs.

In Nigeria, said Ekong, the law now requires majority Nigerian shareholding in most enterprises as well as significant Nigerian representation in management. Nevertheless, technical management is still dominated by the foreign technical partners who determine the technology and the process, including the raw materials, for the industry. Thus the foreign technical partner plays a crucial role in determining the course of industrial development.

Ekong accused these partners of giving biased advice. Their interests as manufacturers, or even their desire to dispose of equipment obsolete in their own countries, will affect their recommendations. Manufacturing processes that require raw materials or key intermediates from the parent company are often chosen.

The Nigerian desire to reduce their dependence on imported machinery and raw materials is thwarted by the lack of provision for research and development. The foreign technical partner provides all the technical know-how and solves any technical problems. Even where the manufacturing process requires quality control involving chemical or physical analysis, many firms use unqualified foreign technicians. Hence, says Ekong, "chemists in developing countries have very few opportunities".

According to A. O. Shobo, of Lever Brothers, Nigeria, however, the situation is slowly changing. While it used to be the case that all R & D problems were referred to headquarters for solution, there is now a gradual recognition that there is a limit to the scope of R & D that can be carried out without an adequate knowledge of local conditions. Local companies are gradually setting up their own R & D activities.

The majority of scientists in Nigeria are in the universities. They have a major role to play in developing local industry based on local raw materials and appropriate technology. But they face many difficulties, the main one being financial. To study a problem until the results are applied in industry requires continual and intimate collaboration between industry and the university which few local enterprises can afford. It is easier to buy imported processes even where this means importing preprocessed raw materials or intermediates.

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Ekong suggested solving this problem by compelling all industrial enterprises in Nigeria to contribute to a fund for local industrial R & D. They would be able to claim rebates depending on how much they spent on local R & D. He also suggested that tax incentives be given to industries based on local knowledge and raw materials.

Ekong believes that without significant input from the universities there will be no self-sustained growth in industrialisation in the foreseeable future. However, because governments regard research facilities as luxuries developing countries can ill afford, "most chemical laboratories in our universities are ill-equipped and lack basic facilities for even the most elementary research", says Ekong. "Lack of scientific leadership is the principal reason for the low scientific output and is an area where international collaboration could be fruitful. Dr C. C. Mjojo from Chancellor College, Malawi, pointed out that there was little difficulty in finding money for applied research but support was needed for the pure natural sciences.

Lynette Hamblin

Lynette Hamblin is editor of Education in Chemistry

Grain production in Pakistan. Top left: terraced farming. Above: grinding wheat.

Pakistan reorganises agricultural research after harvest disaster

AFTER a disastrous wheat harvest in Pakistan last year (the shortfall in expected production was 1 million tons), the Pakistani government has had to import 2.2 million tons of wheat at a cost of 4,000 million Rupees ($400 million). The bad crop came as a particularly hard blow because the country had hoped to achieve self-sufficiency in food last year and wheat is its staple food.

The poor crop can be attributed to the fact that 75% of the wheat was afflicted by rust. The main reason for such widespread infection seems to be due to the fact that the popular commercial wheat varieties used had not been bred for rust resistance.

The Pakistani government lays the blame with its agricultural policy and the way in which the country goes about agricultural research. There are agricultural research institutes in Pakistan but most of them are isolated in the provinces and suffer from a lack of central coordination. Most of their research on breeding has centred on improving yields. Breeding resistance to adverse conditions such as disease or weather has been largely overlooked. And many very important aspects of agricultural research such as climatology, plant biology, agronomy and pathology are barely covered at all.

Dr Amir Mohammad, chairman of the Agricultural Research Council in Karachi has plans to improve Pakistan's agricultural research. The ARC has already drawn up the following measures for quick implementation:

- The creation of a National Agricultural Research Centre. This is already being built in Islambad on 1,300 acres of land. It is planned as a centre of excellence for agricultural research and is being funded by Pakistan and to the tune of $19 million by foreign grants and loans.

  The centre will consist of institutes on agricultural climatology, plant biology, agricultural data and planning, agricultural engineering and machinery, forestry, fisheries, water-shed management, arid-zone research, rural sociology, and a national plan introduction centre. Building should start in mid-1979 and the centre should be functioning in about three years time.
- The creation of a National Commodity Institute under the ARC. This will conduct research into individual crops and livestock. It will consist of separate sections for each major crop, such as wheat, rice, sugarcane, and cotton and a livestock section. Existing provincial research institutes will remain but will be under the NCI and the NARC.
- Manning of the institutes. 150 first-rate scientists will be needed. Pakistan is short of such manpower, so it plans to put new graduates into a six-month training course and then to send most of them for further training abroad.
- The division of the country into eight agro-ecological zones each carrying a different climate and ecology. An advisory committee of scientists and local farmers for each particular zone, will meet periodically to assess local conditions and recommend measures for ensuring optimum output.
- Strengthening of the small cereal Diseases Research Institute operating under the ARC so that it can incorporate studies of new cereal pathogens and screen new cereal strains for resistance to various rusts.
- The development of a coordinated research programme. ARC plans to have a strong research group on the socio-economic aspects of agriculture, which will also deal with large and small-scale planning of the country's agricultural resources.

At a Wheat Research and Production Seminar held in Inslambad last August, Dr Norman Borlaug, Director of CIMMYT, Mexico, expressed the view that Pakistan could increase its wheat production from 8–9 million tons to 15 million tons if farm production was made more efficient. At present, in spite of the 'Green Revolution', the wheat yield in Pakistan is quite low. As against Mexico's 4,175 kg/Hectare, Egypt's 3,343, and the US's 2,022, wheat production in Pakistan is only 1,411 kg/Hectare.

Azim Kidwai

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correspondence

The Piltdown hoax

SIR,—Halstead's preliminary presentation of allegedly new material on the Piltdown hoax[1] could not reasonably be expected to encompass all the evidence which has accumulated in relation to anthropology's most notorious forgery. Nonetheless, one might have hoped that the tape-recorded allegations of the late Professor Douglas would have been accorded a tolerably critical analysis. Such is not the case.

Douglas and Halstead's attempt to implicate Sollas (and Dawson), whilst it may appear plausible to the casual reader, at best represents only a small part of the truth about Piltdown, and at worst depends upon exaggerations and imprecisions. Let me give some examples.

Although Halstead does not qualify Douglas' assertions about Dawson's reputation, they are in fact grossly misleading. Whether or not Dawson was guilty of Piltdown, he certainly did not acquire a reputation for perpetrating hoaxes during his lifetime. In fact he was a respected collector of fossils and antiquities for the British Museum. To be sure, after the exposure of the Piltdown forgery in 1953, a number of insinuations against Dawson's integrity were assembled,[2] and it has recently been shown that some Roman brick-stamps which Dawson donated to the British and Lewes Museums are forgeries.[3] However in the first instance the insinuations may represent retrospective mud-slinging by local Sussex antiquaries jealous of the international fame which Dawson achieved through Piltdown, and in the second instance the possibility remains that Dawson was the dupe rather than the forger. Certainly prior to his death in 1916, there was not a hint of scandal about Dawson. Even in relation to Piltdown itself, Dawson's guilt is far from proven. Of the three full-length books written about Piltdown after 1953, two argue that Dawson was innocent,[4] and the third, while implying Dawson's probable guilt, pointedly avoids expressing its final conclusion in positive form.[5]

Apart from glaring inaccuracies such as the above, the Douglas/Halstead hypothesis embodies many statements which are so vague as to be practically worthless as evidence. The potentially crucial assertions that Sollas knew and visited Dawson, that Sollas received a packet of potassium bichromate, and Sollas borrowed some ape's teeth, are left with no firm indications as to when these events allegedly occurred. If they took place after December 1912, their occurrence would almost certainly be irrelevant to the hoax. Even the incident of the Sherborne Horse's Head, which constitutes the most suggestive part of the Douglas/Halstead case, is open to many interpretations.

Certainly a Sollas/Dawson conspiracy would provide a possible, perhaps even a likely, explanation for the Piltdown hoax, but the best evidence for such a conspiracy lies in areas untrodden by Douglas and Halstead. The 1911 edition of Sollas' *Ancient Hunters* provides a detailed description of "Heidelberg man", an earlier candidate for the title of "missing link" which probably served as a model for Piltdown, and which is known to have preoccupied Dawson circa 1912. Moreover, since the Piltdown forger chemically treated the cranial fragments in an attempt to make them appear fossilised,[6] it is surely worth noting that Sollas had done important pioneering work on the chemistry of fossilisation,[7] work which Dawson may well have utilised in interpreting the Piltdown remains.[8]

However, because of problems surrounding Sollas' motivations, problems which Halstead's communication fails to resolve, I am inclined to lay the blame for Piltdown elsewhere. My current working hypothesis is that Elliot Smith instigated the hoax, and that Smith Woodward may have been a willing accomplice. This hypothesis does much to illuminate a formerly puzzling episode from Australian prehistory, an episode in which, incidentally, Sollas played a supporting role.[9]

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Allies against Lysenko

SIR,—In Vera Rich's moving account of Professor Lev Tumerman's seven years in solitary confinement (14 December, page 662), she mentions that he recalls "one very good book by Sunnot and Dent". He no doubt meant to refer to the classical textbook of genetics by Sinnott, E. W. and Dunn, L. C. *Principles of Genetics*, McGraw-Hill, New York and London, 1925 which went through several editions (the last with Dobzhansky) and which for almost four decades (1925–1965) was the standard text in this field. E. W. Sinnott was professor of botany at Columbia and then Yale and L. C. Dunn was professor of zoology at Columbia. Dunn, who died in 1974, was active in the field of developmental genetics for more than a half-century; a fascinating review of his life and work has appeared recently (Bennett, D. *Ann. Rev. Genet.* **11**, 1–12; 1977).

It is interesting that Dunn, who was often bitterly attacked in the United States for his devotion to what were at that time left-wing causes, was an outspoken critic of Lysenko and of the Soviet persecution of their geneticists and of other scientists and intellectuals, such as Tumerman himself.

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Who assesses risk?

SIR,—I was much struck with Wendy Barnaby's report of the Swedish seminar on risk assessment (7 December, page 554). The impression conveyed was that informed and impartial politicians ought to regard a democratic society's evaluation of risk perception as even more important than quantified objective risk. This is tantamount to saying that it is more important to consider the number of people who would be frightened than the number of people who would be killed.

The conclusion is that one should favour energy production from fossil fuels which kill far more people, rather than nuclear power which frightens far more people.

I can see why short-sighted politicians might approve this view, assuming that the fears of the public will last at least until the next election. Dead people don't have votes. Frightened people do. But I am surprised that *Nature* appears to approve the same opinion.

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Not by force of ARMS

SIR,—I welcome the article by Joe Schwartz (23 November, page 310), drawing attention to the unsatisfactory job security and career prospects of research staff. Technicians and graduate research assistants, as well as post-docs, are often victims of short term contracts.

May I underline the point that it is the quality and strength of local trade union organisation which determines how effectively these issues can be negotiated with each university, medical school, college or institute. For this reason I was alarmed by the suggestion that ARMS "intends to negotiate with employers". Indeed, I am sure it would delight these employers to be relieved of the embarrassing task of trying to justify their practices to the experienced negotiators of strong trade union organisations. I welcome ARMS to the extent that its existence demonstrates the concern felt by research staff, but I must qualify this by warning that the ARMS could backfire if it diverts staff from the need to work within trade unions.

Finally, I should clarify that I am not a "division organiser" for ASTMS (as reported in your article). I am one of many lay activists within ASTMS who have been tackling these issues, and many others, for some years.

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London, UK.

news and views

Quaternary correlations

from D. Q. Bowen

For the foreseeable future the major challenge confronting Quaternary research must be correlation of the oceanic and continental records. Continental models of Quaternary history are now inadequate and already a re-evaluation has commenced. Astonishingly this has been long delayed, for the implication of such inadequacy, and of greater complexity was plain for all to see from Emiliani's work on stable isotopes from deep sea cores during the early 1950s. Partly the delay was due to misconceived interpretation of the oceanic evidence in the light of continental models, whereas now, despite some lingering opposition, the horse has at last been placed firmly in front of the cart.

Notwithstanding the eminently reasonable geological suspicion of notions of continuous sedimentation, the open ocean (pelagic) environment constitutes a special case. There sedimentation by settling through the ocean takes place more or less constantly for long periods as demonstrated by inter-core correlation throughout the world's oceans based on repetitive isotopic signature, palaeomagnetic character, assemblage faunal analysis and, increasingly, by the extinction of certain taxons at isochronous levels.

The oceanic oxygen isotope stratigraphic framework shows the changing isotopic composition of the world's oceans throughout the Quaternary. Preferential evaporation of the lighter ^{16}O and its incorporation into ice sheets on the continents tends to enrich the oceans residually in ^{18}O during glacials. Conversely during interglacials isotopically lighter water is returned to the oceans. Foraminifera from deep sea cores, which precipitated their calcareous tests in isotopic equilibrium with the water they inhabited, monitor such changes, and allow definition of oxygen isotope stages numbered backwards in time, using odd numbers for interglacials and even ones for glacials. This stratigraphic framework records changing ice volumes on the continents, and by inference the changing climate also. It shows that during the 1.6 Ma of the Quaternary some 18 glacials occurred which compares with the celebrated four-fold glaciation model of the Alps. This has dominated thinking for so long although it only represents the last 850 ka during which time 10 actual glacials occurred. The extent to which the Alpine model influenced others and the terms on which new data were collected and evaluated in terms of a four-fold sequence, is only now becoming apparent with the realisation that much of the primary data base in terms of basic field geology is inadequately known in many areas.

Without geochronometric control, for example ^{14}C, uranium series and K/Ar, traditional means of correlation continue by plotting data graphically to show fluctuations of climate through time. Correlation is principally by tele-correlation relying on curve-matching assisted occasionally by radiometric tags at crucial points. Hitherto the best results, which bear an amazing resemblance to the oceanic record, come from the loess terrains of central Europe (with signs that work on the loess of China is not far behind). There, sequences of loess, palaeosols and other indicator deposits, coupled with the recognition of palaeomagnetic reversals for dating, allow correlation back to the beginning of the Pleistocene. Other key localities include the long lake sequences of the Vosges, Macedonia, Colombia, Queensland and Japan, where climatic variations are recognised from pollen analysis. Such a technique is eminently successful when applied to continuous layers of sediment, but rather less so when the data are fragmentary and stratigraphic succession inferred by an additive process using geographically widespread data. One pollen sequence may look much like another for the fact is that repeated constellations of species re-appeared during successive similar climatic episodes, perhaps with different emphasis in the relative timing of arrivals, but as the regional variation of the vegetation is largely unknown this cannot be used for definitive dating. For Northern Europe it was formerly thought that the interglacials of the Middle and Upper Pleistocene were known and sufficiently well differentiated—Cromerian, Holsteinian and Eemian. However, in the light of the oceanic evidence, there must be at least six interglacials. Clearly the problems are beyond the resolving power of the method, which is not surprising since it relies not on first appearances nor extinctions, but on assemblage floras, a biofacies basis which is inherently unreliable on account of its diachronous nature.

Using this method marine deposits from Western Norway are correlated with the Eemian Interglacial and also with oxygen isotope stage 5e (Mangerud *et al.*, this issue of *Nature* page 189). In equating Eeemian with stage 5e the authors follow Shackleton (*Proc. Roy. Soc. Lond.* **B.174**, 135; 1969) and there can be little doubt that the Last Interglacial at 125 ka is a prominent feature of sequences throughout the world. The chrono-

Oxygen isotope stage	Classical continental stages		
1	'Holocene' (postglacial)		
2			
3	Weichselian (last glaciation)		
4			
5a-5d			
5e	'Eemian' (of Schleswig-Holstein)	Eemian sl	Saale sl
6	Warthe		
7	'Eemian' (northern Germany)		
8	Reburgh		
9	'Eemian' (Amersfoort)		
10	Saale ss		

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stratigraphic term Eemian, however, is potentially misleading, for Kukla makes a compelling case for the recognition of three stratigraphically separate Eemian Interglacials (*Earth Sci. Rev.* **13,** 307; 1977). The type Eemian, considered by the authors, is near Amersfoort in the northern Netherlands. It lies between Saale glacial deposits and sands of the Last (Weichselian) Glaciation (isotope stages 2, 3, 4 and 5a–5d). Kukla argues that three glaciations occurred during the period traditionally referred to as Saalian (Saale(ss), Rehburg and Warthe), and that the Saale (ss) corresponds to oxygen isotope stage 10. Thus because the Amersfoort Eemian deposits appear to be continuous with the underlying Saale(ss) they should be correlated with stage 9. Eemian of isotope stage 5e is probably represented by the interglacial deposits of Schleswig-Holstein where marine beds clearly overlie and breach end-moraines of the Warthe Glaciation (isotope stage 6). Clearer stratigraphic demonstration of two Eemian Interglacials (probably corresponding to stages 5e and 7) is found in Northern Germany where they are separated by glacial deposits (Wiegank, *Geologie* (*Berlin*) **21,** *Beih.* **77,** 1; 1972).

Correlation of the Norwegian deposits with the type Eemian (stage 9?), on floristic grounds, and also with stage 5e, is, therefore, not unequivocal. It may be that correlation with the Eemian of Schleswig-Holstein (5e?), is sounder, because faunal evidence from the Norwegian deposits, showing higher temperatures than today, matches stage 5e from deep sea cores in the Norwegian Sea. Such a correlation is not as tenuous as might appear for comparable temperatures have only been recorded twice in the last 450 ka.

The problem of 'Eemian', and other similar terms, remains, and is symptomatic of the state of continental frameworks at the present time. These require urgent revision and redefinition in the way Kukla has attempted for Europe. It may mean a plethora of new stage terminology, often on a local if temporary basis, but until the basic ground is secured in this way progress will be hindered. The existing continental frameworks cannot continue to bear the brunt of attempted correlation with oceanic or other records either for the specialist or numerous users of Quaternary data. ☐

Bacteriophage assembly

from Roger W. Hendrix

Any recent textbook of cell biology or biochemistry contains a diagram based on the experiments of Edgar and Wood that outlines the assembly of bacteriophage T4. In the best tradition of Henry Ford, protein and DNA molecules assemble sequentially into the component parts (heads, tails, tail fibres), and these subassemblies then join each other to form the finished product, the virion. This scheme of phage assembly has been incorporated into biochemical lore, widely quoted, sometimes overinterpreted, and not infrequently taken to represent a relatively complete understanding of virus assembly. However, despite the elegance and importance of Edgar and Wood's original experiments, basic questions are still unanswered, and phage assembly remains a fertile field for investigation into the principles of biological form determination. Some of the highlights of recent work in the field were aired last September at the Sixth Biennial Conference on Bacteriophage Assembly.

Forming the intellectual backdrop to the meeting were a number of major conceptual advances in understanding virus assembly that have developed over the past decade. Some of these are the following. Assembly pathways are in general quite strictly ordered and, although all of the relevant proteins are present together in the cell, they associate with each other only when the pathway requires it. In other words, the order of assembly is regulated by the assembly pathway itself rather than, for example, by the order of transcription or translation. Virus assembly, at least in the case of the structurally elaborate phages, is more than a simple 'self-assembly' process in which all of the information for assembly resides in the components present in the final assembled product. Although many individual steps in the assembly of these viruses undoubtedly involve true self assembly, other steps in the process require the intervention of proteins that act as assembly tools or catalysts and are not themselves found in the completed virion. Among the 'tools' that are beginning to be characterised ~~are~~ proteins that form transient scaffolds which direct the assembly of other proteins, and enzymes that cause covalent alterations of proteins already assembled. The actual DNA packaging event has been a central issue for many years, and it has become clear that DNA is condensed into a preformed protein shell—this in contrast to the original idea that the DNA first condenses and is then surrounded by protein.

Several of the 50 papers presented in Toronto dealt with the question of size determination. That is, how is it that virus substructures such as heads and tails are virtually always the right size, even though their component proteins can frequently be shown *in vitro* or in mutants to be capable of forming structures of a variety of different sizes? An interesting case is found in *Escherichia coli* phages P2 and P4. P2 by itself makes uniform sized heads. However, if a cell is coinfected with P2 and P4 (a satellite phage which itself codes for no head protein) then the P2 head proteins are assembled into smaller P4-sized heads. Assembly of P2 proteins into P4 heads is directed by a P4 gene that has been named *sid* (*s*ize *d*irection). J. Geisselsoder, M. Chidambaram, and R. Goldstein (Harvard University) presented evidence that the *sid* gene influences the efficiency of transcription termination on the P2 genome. They proposed that in the presence of the sid^+ gene product the relative amounts of certain P2 head proteins are altered, and that it is these crucial ratios that determine the size of the head assembled from the head proteins.

In the case of size determination of T4 heads, interest centred on the well known fact that the head is elongated in one direction. Genetic studies reported by D. Doherty (University of Washington) show that mutations in the gene coding for the major head protein (gp23) which cause the production of non-elongated heads can be suppressed by simultaneous mutations in genes *22* and *24*. These genes code for the protein (gp22) of the scaffold around which gp23 assembles and the protein (gp24) that constitutes the corners of the head. The results suggest that the three proteins interact with each other to determine head length. L. Onorato and M. Showe (University of Basel) presented biochemical experiments which also implicate these three proteins in head length determination. Their experiments further suggest that the ratios among the concentrations of these proteins influence the size at which the growing shell is capped off; thus they arrive at a conclusion analogous to the results with P2 and P4 heads by a different experimental route. R. van Driel (University of Basel) described reconstitution experiments with purified proteins which showed that the scaffold protein (gp22) will assemble in the presence of an initiator protein (gp20) into what appear to be normal

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sized scaffolds. This argues that at some level the scaffold protein 'knows' the correct head length even in the absence of gp23 and gp24.

The mechanism of DNA packaging has been a favourite problem in the field, and there has been impressive progress in recent years, particularly since it has become possible to package DNA *in vitro*. The papers on DNA packaging in Toronto ranged over a wide variety of topics including how the DNA is recognised by phage proteins, how it is cut to its mature form, and the relation between cutting and packaging. One particularly interesting report for this field which has primarily been concerned with packaging double-stranded DNA, was a paper by K. Koths and D. Dressler of Harvard University on packaging the single-stranded DNA of φX174. Their evidence shows that the preformed protein capsid is associated with the replicating DNA, apparently as part of a complex at the replication fork of a rolling circle, where it takes up the DNA as it is made. Replication and packaging of DNA are tightly coupled in this case, and it is tempting to speculate that the driving force for packaging might be derived from replication. This is in marked contrast to the case of double-stranded phages where replication and packaging are clearly separable.

Two models were presented for how DNA is moved into the heads of double-stranded DNA phages (L. Black, University of Maryland; R. Hendrix, University of Pittsburgh). These, if nothing else, reflect the current state of experimental evidence on the problem, they both differ from previous models in postulating that the force that drives the DNA into the head is applied by a small proteinaceous machine that sits in the head shell at the point where DNA enters (thought to be the point at which the tail later attaches).

The most stimulating paper at the meeting, to my taste, was one by J. Shaw and H. Murialdo (University of Toronto) which admits phage lambda to the club of viruses with overlapping genes. They find that the lambda head gene *Nu3* is contained within the last one-third of the *C* gene. The two corresponding proteins, gpNu3 and gpC, are read in the same phase and presumably use the same translational termination signal (that is gpNu3 is identical to the C-terminal one-third of gpC). The real interest in these results probably lies not in the fact that they give yet another example of overlapping genes but in the fact that it may be relatively easy to decipher the functional significance of the overlap. gpC is most probably part of the head initiation complex on to which the scaffold protein gpNu3 assembles, and it is easy to imagine models in which the C-terminal portion of gpC might serve, for example, to nucleate the assembly of the gpNu3 scaffold.

Much of the research in phage assembly in past years has dealt with working out pathways, measuring compositions of intermediate structures, and otherwise defining the outlines of assembly processes. It was evident in Toronto that the emphasis is now moving toward more detailed mechanistic questions at the level of how proteins and parts of proteins interact with each other and with nucleic acids. For example, two elegant structural studies addressed the question of how the protein subunits of the head shell change their conformation as the shell expands during DNA entry. S. Casjens (University of Utah) used freeze-etch electron microscopy to argue that the major head shell protein, gp5, of P22 consists of two domains and proposed that head expansion could be accomplished by the flexing of a hinge connecting the two. A group from the University of Basel (U. Aebi, R. van der Broek, P. R. Smith, B. ten Heggeler, J. Dubochet, V. Mesyanzhinov) examined the analogous lattice expansion in T4. By using electron microscopic image processing techniques and antibody fragments, they were able to visualise a specific antigenic site on the T4 major head protein gp23. They found, surprisingly, that on lattice expansion this site moves from the inside to the outside surface of the head.

The Toronto meeting was noteworthy for the consistently high quality of the papers, most of which have necessarily been skipped over in this report. It was particularly gratifying to see that for a number of the interesting problems of phage assembly there is now a large body of information from each of several phages. This has made it possible to begin to decide which features of a given assembly process are fundamental and which are variations on a theme. The general impression was that the groundwork for understanding assembly of phages has been solidly laid and that future work will be progressively more concerned with problems that can be generalised to other biological systems.

Muons and muon trapping by solid-state imperfections

from A. M. Stoneham

THE positive muon has an obvious potential in solid state physics. Its spin polarisation can be monitored to give data such as the site it occupies, local magnetic fields, diffusion rates and Knight shifts. If a suitable beam is available from a meson 'factory', the experiments themselves do not need especially sophisticated equipment. So it is not surprising that the μSR field is very active (see *News & Views* **276**, 324; 1978) both in an attempt to understand the way muons behave in solids and to exploit muons to understand better the solids in which they decay.

Several groups have found evidence that muons can probe defect and impurity properties in metals. Since the defects include deformation-induced dislocations and damage from neutron irradiation, and since the impurities include both alloying metals and trace impurities like oxygen, the muon promises to be a very versatile tool. Many gaps remain. To understand these, one must look at the principal processes involved. The first is the slowing-down process in which the muon comes to rest. Usually the muon will end in an interstitial site, self-trapped by the lattice distortion it causes, and vibrating like any normal chemical impurity. The second process is diffusion. Presumably this is described by the quantum theory developed for light interstitials by Flynn and Stoneham; the features of importance here are that the rate should increase with temperature, as a power law at low temperatures and thermally-activated at higher temperatures. One might expect μ^+ diffusion to resemble diffusion of H^+, D^+ or T^+, with a suitable isotope effect, though present data are too limited to check in detail. The third process is defect trapping, due to the strain fields of imperfections or to their perturbation of the electronic structure. At high temperatures, release from traps occurs. There is also a fourth process, independent of the solid: the muon decays with a characteristic time of 2.2 μs, and this imposes limits.

If there is only a single type of trap, one can outline the way the depolarisation rate should vary with temperature. Four main regimes are expected. In regime I, at the lowest temperatures, the muon does not diffuse significantly; it merely comes to rest, and measures an average property of the solid. In II

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Primaeval magnetic monopoles

from P. C. W. Davies

One of those curious apparent imperfections of nature is the lopsidedness of electricity and magnetism. The world abounds with electric particles (all atoms are made of them) but nobody has ever seen a pure magnetic particle Magnetic fields can, it seems, only be produced by moving electricity, that is, electric currents Consequently, when magnetism occurs, magnetic charge always appears in tandem (north and south), never singly Tandem charges are called dipoles and single charges, if they exist, are known as monopoles. Matter is built out of subatomic electric monopoles, but magnetic monopoles elude us. This is all the more surprising because of the celebrated mathematical symmetry built into our theory of electromagnetism.

The great nineteenth century physicist James Clerk Maxwell unified electric and magnetic fields, and discovered the principles which govern their interplay, but his beautiful theory is marred by the asymmetry of the monopoles. In the 1930s Paul Dirac gave an argument, subsequently developed by Julian Schwinger, that if magnetic monopoles exist they will have magnetic charge only in fixed multiples of a fundamental unit, simply related to the fundamental unit of electric charge, such as we find on the electron A few years ago the Soviet and Dutch physicists A. M. Polyakov and G 't Hooft argued that magnetic monopoles could exist on subatomic particles with masses in excess of five thousand proton masses.

There has always been room in theory for magnetic monopoles, but elaborate searches among ordinary matter have persistently failed to detect any. Despite a recent flurry of excitement that one had been found in cosmic ray debris, the monopole remains perhaps the most significant non-discovery of the century.

Sometimes negative evidence can be useful in revealing information about other physical systems. The non-detection of quarks, for example, has been used as a motivation for theories of subatomic particle structure In a recent paper (*Phys Lett.* **79**B, 239; 1978) the Soviet mathematical physicist and astronomer Ya. B. Zeldovich, and a colleague at the Institute of Applied Mathematics in Moscow, M. Yu. Khlopov, have argued that the experimental absence of magnetic monopoles raises serious doubts either about their existence or the popular understanding of the origin of the Universe. The central idea is the theory that the Universe began with an intensely hot big bang. Indeed, so energetic was this event that we can be sure that any subatomic particles that can exist would have existed at sufficiently early moments (that is, sufficiently high temperatures) And this includes magnetic monopoles, despite their predicted enormous masses

Zeldovich and Khlopov calculate that inside the first 2×10^{-11}s the monopoles would have been in thermodynamic equilibrium at a certain concentration among all the other matter, but that as the explosive expansion rapidly cooled the primaeval cosmological material, the temperature fell too low to sustain the monopoles, and they started to annihilate each other. The annihilation is a consequence of fundamental physics that a magnetic north pole is the antimatter image of a south pole, and close encounters between them result in mutual destruction.

In the subsequent phases of the expanding fireball, most of the monopoles would have disappeared as a result of this mechanism But not all. Merely on statistical grounds some monopoles would have avoided their opposite numbers and escaped this demise. This is because both the density of matter, hence monopoles, was falling rapidly, and the temperature, hence speed, of subatomic particles also plummeted. The authors calculate the residue of relic monopoles coughed out of the big bang which should still exist. The annihilation ceases after about 10 ms, leaving a total concentration today of around 4×10^{-19} cm^{-3}, or less than one monopole per million million atoms Small though this is, it is enormously greater than the experimental upper limits already set ($10^{-19}-10^{-28}$ cm^{-3}) from examination of lunar and terrestrial matter, and cosmic ray studies.

There seem to be two alternative explanations of this inconsistency. One is that nature truly is lopsided, and that magnetic monopoles simply do not exist in isolation (There is still the possibility that for some obscure reason monopoles exist in permanently confined multiplets, like quarks) The second is that the standard hot big bang model of the Universe is badly wrong, at least in some of its details We thus have an additional motivation for seeking out monopoles, for their discovery as free particles would, unless the previous abundance estimates are a fluke, tell us much about the first micromicrosecond of the Universe.

One of the ironies about papers of this type concerns the central role played by aesthetics Belief in magnetic monopoles stems primarily from a deep-rooted desire that nature should be elegant and symmetrical. Similarly in cosmology, sweeping assumptions are made about the structure of the primaeval Universe which, once again, stem from the yearning for simplicity Now we see that cosmic simplicity (primaeval thermodynamic equilibrium) and subatomic symmetry are in conflict The resolution cannot be found by aesthetics alone. Only experiment can decide which assumption is wrong

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the muon diffuses faster and faster as the temperature rises, and the rate at which it samples different internal magnetic fields affects the depolarisation rate and can be measured. This regime ends at a temperature fixed by the trap density, and in region III, the muon monitors properties of the trapping site. Finally, in region IV, release from traps and motional-narrowing occur Obviously, behaviour gets more complicated when several distinct traps are involved, but the principles are the same. Many workers have obtained results fitting this qualitative description of the temperature dependence.

For this picture to be useful, it is important that the muon can indeed diffuse to the traps of interest. The muon lifetime sets a limit here. In most metals studied (V, Nb, Ta, Cu, Fe) at accessible temperatures the muon hopping time between diffusive jumps is in the range 10^{-8}–10^{-7} s corresponding to a mere 0.2 to 20 jumps in a lifetime. Diffusion from a random stopping site to any trap present in low concentration is negligible. And indeed the irradiation of Cu and the doping of Nb by Ta have little observable effect on muon properties Two metals are different, and appear to show very fast diffusion : Cr, with a hopping time around 10^{-11} s and Al, where motional narrowing appears to be complete even at the lowest temperatures, with times such as 10^{-10} s at 10 K and 10^{-12} s at 50 K indicated.

Several groups have studied trapping

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in Al, including workers at laboratories in America, Sweden and Germany. Two results are particularly interesting. One was reported by Seeger at a recent Rutherford Laboratory meeting, and is from Stuttgart workers, who looked at neutron-irradiated Al. The point here is that the basic defects, and hence the traps, can be modified by annealing. Reversible changes with temperature reflect the muon motion, whereas irreversible ones indicate changes in defect structure and can be related to previous damage work. Two traps were detected in Al : one was shallow, possibly an interstitial cluster; the other trap was deeper and is not yet identified. The second result comes from a collaboration among five of the American laboratories (*Phys. Rev. Lett.* **41,** 1558; 1978). The experiments showed the effects of Cu impurity and of deformation. The dislocations act as deep traps, with a binding of around 0.2 eV, and also seem to show the rapid pipe diffusion known from conventional diffusion studies. The Cu appears to give rise to some four different shallow traps (binding the muon by less than 1/50 eV) even at the low concentrations used (420 and 1,300 p.p.m.). The authors argue these traps are microclusters of Cu, and that the clusters are present at levels far above random. If so, the muon technique is giving new information about alloy structure in circumstances hard to match otherwise.

What then are the prospects? If the interpretations are right, muon spin resonance adds another tool to the study of alloy structure, complementary to positron annihilation, neutron scattering and various forms of electron microscopy. Clearly muon experiments need a large accelerator, and cannot be as portable as techniques like positron annihilation; applications to macroscopic defect structure, as in nondestructive testing, will have to be reserved for special cases. There are also some open questions. Are there really so many microclusters in Al:Cu? Are there systems where such muon results can be checked by other methods, like magnetic or Mössbauer techniques? If these microclusters are real, do they affect mechanical properties? Another question is in how many systems can the muon probe defects and impurities? Diffusion rates mentioned earlier suggested that the success with Al was the exception, not the rule. This need not be so. Slow diffusion only prevents the muon probing a trap if the stopping site is random within the crystal. However, a slowly-moving muon may be stopped preferentially near defects, by its interaction with their strain field or otherwise. This may be why results on vanadium are very sensitive to interstitial oxygen. In such cases the muon will probe the defects, but will give little information about the perfect host. Non-metals present their own special problems. Ionisation-enhanced diffusion in semiconductors may ensure the muon can always reach a defect; recombination-produced vacancies and interstitials in ionic crystals may ensure there are always defects to be reached. All in all, the exploitation of muons promises to yield scientific problems as well as solving materials ones. ☐

A hundred years ago

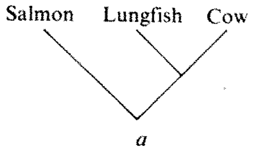
INVALIDS will be glad to learn that, amidst the severe weather, a comparatively mild climate exists in a part of their own country. A bouquet arrived from Glengariff on Saturday, the 11th instant, comprising wallflower, primrose, primula, stocks, chrysanthemum, scarlet geranium, arbutus-berries, and a rose-bud, all picked from an exposed garden. In Madeira—but five days' journey—ripe bananas and custard-apples are hanging on the trees. Many of the gayest flowers are in full bloom.
From *Nature* **19,** 16 January, 257; 1879

The salmon, the lungfish and the cow: a reply

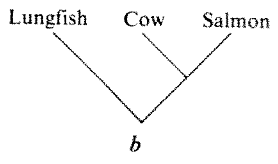
THE debate on cladism has smouldered in specialist journals for over a decade, and we are glad that Halstead has brought it to *Nature* (*News & Views* **276,** 759; 1978). An adverse review (Cain *Nature* **216,** 412; 1967) of Hennig's book (*Phylogenetic Systematics* University of Illinois Press, 1966) is about the only previous sign of the controversy in this journal. As participants in the meeting at Reading, named in Halstead's report, we find his account seriously defective in two respects—as a description of the meeting, and as a summary of the claims of cladism and the history of the controversy.

On cladism in general, Halstead's report reveals several assertions and misunderstandings about aims and methods that were common in the early days of the controversy, and have been fully discussed in *Systematic Zoology* and other journals. Rather than repeat those arguments, we should like to focus on what is, in our view, the principal source of acrimony between cladists and evolutionary systematists, a problem neatly summed up in a question raised at the Reading meeting and reported by Halstead: 'according to the cladists a lungfish is more closely related to a cow than to a salmon'. Halstead correctly reports the exasperation with which the accusation was put, and the complacency with which one of us accepted it. If experienced comparative anatomists can disagree on so simple and fundamental a question, either there is revolution in the air, or words are being used in different ways.

Given three taxa—for example, a lungfish, a salmon and a cow—a cladist will draw up the three cladograms showing the three possible 'more closely related' pairs.



a



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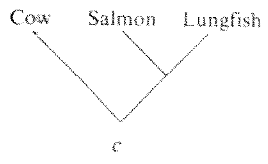

c

Each of these concepts has its advocates, but at Reading, *a* was the preferred solution of the cladists, us included, and *c* was the preferred solution of Parrington and Halstead, whom we shall refer to as evolutionary systematists, following Mayr (*Z. Zool. Syst. Evolforsch.* **12,** 94; 1974). Since we do not believe that evolutionary taxonomists disagree with us over the characters of lungfish, salmon and cattle, the disagreement is evidently over the meaning of the concept 'relationship'. Although 'relationship', 'affinity' and cognate words have been freely used in the evolutionary and pre-evolutionary literature of biology, Hennig was the first, to our knowledge, to give an unambiguous definition of the concept. Mayr (*op. cit.*) summarised Hennig's definition of relationship; 'the measure of phylogenetic relationship is the relative recency of common ancestry'. Stung to give precision to a concept they had previously used in various senses, evolutionary systematists seem to have settled on 'genes in common' as the measure of phylogenetic relationship (Mayr (*op. cit.*) 'inferred amount of shared genotype'). Ignorant as we are of the correspondence between the genotype and phenotype, and of the genotype in the vast majority of organisms, 'genes in common' is a rationalisation of 'characters in common', or overall similarity. Hence, when evolutionary systematists prefer cladogram *c*, they mean that lungfish and salmon are more similar than lungfish and cows. And in preferring cladogram *a*, cladists mean that they infer that lungfish and cows shared a more recent common ancestor than lungfish and salmon. This inference is drawn from the fact that lungfish and cows share derived characters (synapomorphies such as internal nostrils, an epiglottis, a two-chambered auricle and so on (*Kesteven Proc. Roy. Soc. Vict.* **59,** 93; 1951)) not found in salmon.

Given their reliance on overall similarity, it is not surprising that evolutionary systematists find that many of the groups they recognise are grades, nor that these grades 'happen to coincide with the major classes of the vertebrates' (Halstead *op. cit.*). Yet these vertebrate classes were recognised and named by pre-Darwinian systematists, who also used overall similarity as their guide, naturally enough. Overall similarity may be modernised or dignified by calling it 'genes in common' or 'shared genotype', yet when we do have access to comparative information on genes, as in globin sequences (Goodman *et al. Nature* **253,** 603; 1975; Romero-Herrera *et al. Nature* **261,** 162; 1976), the biochemists present their information in the form of cladograms, and use the same genealogical concept of relationship as Hennigians. Darwin (*Origin of Species*: see also Nelson *Syst. Zool.* **23,** 452; 1974) wrote 'our classifications will come to be, as far as they can be so made, genealogies; and will then truly give what may be called the plan of creation'. He might be surprised that after 120 years, some palaeontologists would be occupied with the defence of pre-Darwinian concepts and classifications.

Halstead's report contains many misunderstandings and ambiguities, a few of which cannot be allowed to pass unchallenged. It is not, as Halstead says of cladistics, "axiomatic that . . . sister groups are given identical ranks"; it is however a logical consequence if a formal classification is required. Nor does cladistics insist on a classification which reflects all of the ideas embodied in the cladogram. Cladists do not assemble characters subjectively into a hierarchy: though we suspect that the hierarchy of which Halstead speaks is a reference to character weighting, not favoured by cladists. Halstead also accuses cladists of belittling the significance of parallelism. But this is outside the scope of cladism since the recognition of such an evolutionary event presupposes that we already know the phylogenetic relationships of the organisms concerned.

Concerning Halstead's report of the meeting itself, we were surprised to read that papers delivered by two of us 'were demolished within hours'. Freedom of speech goes a little too far here, and we request that Halstead justify his assertion by saying who did the demolition work, and how it was done. Halstead charges us with 'religious fervour', but the charge might be reversed, for to us his statement that cladists 'are already entrenched in some of the major museums in the world' has an offensive taint of McCarthyism and the witch-hunt.

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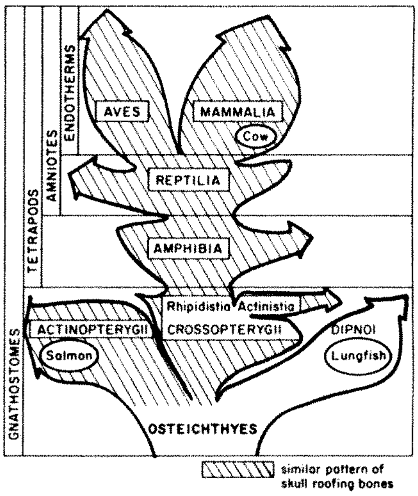
L. B. HALSTEAD AND COLLEAGUES REPLY: The mutual relations of salmon (an actinopterygian), lungfish (a dipnoan) and cow (a mammal, a class which can be traced back to rhipidistian crossopterygians by way of reptiles and amphibians) are certainly worthy of serious consideration. Gardiner *et al.* draw up "three cladograms showing three possible 'more closely related' pairs". Cladogram *a* is their preferred solution, but it is certainly not true to claim, as they do, that "*c* was the preferred solution of Parrington and Halstead". The cladists simply omit the possibility that none of the three pairings shared a more recent common ancestor, than any of the others, but rather were all derived from a pre-existing common stock, a view previously commended by Miles (*Palaeozoic Fishes,* 1971).

Assuming that cow, salmon and lungfish were derived from such a common ancestral stock, we would still insist that lungfish are closer to salmon on the grounds of neither having advanced significantly beyond the common heritage of all primary aquatic jawed vertebrates. In marked contrast, the cow is separated from both lungfish and salmon by several structural grades. The figure summarises these relationships.

At the meeting Gardiner presented a cladogram which purported to demonstrate that lungfish were more closely related to tetrapods than were either coelacanths or certain rhipidistian crossopterygians. The near structural identity of crossopterygians and early tetrapods makes it impossible for them to be separated by the uniquely specialised lungfish. Later in the meeting T. S. Westoll demolished this particular cladogram and pointed out that most of the features that Gardiner claimed were absent in coelacanths were in fact present in the material Westoll himself was studying.



Janvier's agnathan cladogram was a reflection of Stensiö's mistaken notion of the myxinoid affinities of the heterostracans, which has been discussed and firmly rejected by workers in the field (see *Biol. J. Linn. Soc.* **5,** 339–349, 1973; *Biol. Rev.* **48,** 279–332, 1973, for a summary of this issue).

The current Hennigian orthodoxy brings to mind the situation at the beginning of the present century. Then Haeckel's Biogenic Law and the attendant preoccupation with genealogies, had a stultifying influence on the whole of biology. 'When the results of a science are produced by methodological assumptions rather than by the evidence available, it is not science' as A. J. Cain remarked in his review of Hennig's book in *Nature.*

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review article

Cellular interactions in haematopoiesis

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In vitro *culture of haematopoietic cells has provided some surprising insights into critical interactions of blood-forming cells. Subpopulations of lymphoid cells have been shown to produce colony-stimulating activity, to interact with macrophages, and to have important effects on the very early stages of erythropoiesis. Macrophages have multiple influences on the proliferation and differentiation of other haematopoietic cells.*

PRIMITIVE pluripotent haematopoietic stem cells have the capacity to form all the blood elements, including myeloid cells, erythrocytes, and immunocytes[1]. In mice the stem cell for erythroid and myeloid lines is assayed by its ability to form haematopoietic spleen colonies in irradiated syngeneic recipients (hence, the stem cell is often called CFU-S, colony forming unit-spleen). The derivation of colonies from a single precursor cell was established by using a mouse with chromosome abnormalities as the cell donor. The same abnormalities were found in replicating cells of lymphoid tissues diffusely distributed in the spleens and lymph nodes of the recipient mice. Further evidence was derived by retransplantation of non-immunocompetent spleen colonies. Colonies developing in secondary host animals contained immunoglobulin-producing cells with the T6 chromosome marker of the original bone marrow donor. Direct evidence for a single stem cell common to the myeloid and lymphoid lines has recently come from reconstitution experiments in mice in which chromosome markers were induced in donor bone marrow cells by irradiation[2]. Myeloid spleen colonies and T and B lymphocytes in reconstituted animals had the chromosome marker of a common precursor. The appearance of the Philadelphia chromosome in cells of the granulocytic, mononuclear phagocytic, erythroid, and megakaryocytic lines of patients with chronic granulocytic leukaemia is evidence for the existence of pluripotent stem cells in man[3,4].

The process of commitment of pluripotent stem cells to one or another line of development is poorly understood. For example, the pluripotent stem cell is not responsive to erythropoietin but at some point in development it gives rise to an erythropoietin-responsive stem cell. Such a cell is unipotent and exclusively committed to maturation along the erythroid pathway. An analogous situation holds for granulopoiesis and responsiveness to colony-stimulating activity. In a certain sense, commitment may be defined as the acquisition of responsiveness to a specific haemopoietin. Under the influence of specific haemopoietins, there is further cellular proliferation and maturation until appropriate numbers of functional end-cells are produced. It is likely that in physiological circumstances most haematopoietic regulation occurs at the level of the committed stem cells. It has become apparent in the past few years that a diversity of cellular interactions can influence the formation of mature blood cells from stem cell precursors. The characterisation of these interactions had to await methodology for *in vitro* culture of haematopoietic cells. Of particular importance has been the development of a semi-solid gel culture system[5,6] for the growth of monocyte stem cells and of granulocytes (CFU-G,M). (In this review the term granulocyte is taken to mean neutrophilic granulocyte, although, strictly speaking, it encompasses neutrophilic, basophilic, and eosinophilic granulocytes.) With the subsequent development of *in vitro* techniques for assaying erythroid, megakaryocyte and eosinophil precursors and B and T lymphocytes[7], characterisation of the haematopoietic hormones which influence proliferation and differentiation has become possible. More recently, these techniques have been used to explore cellular interactions of combinations of purified cell populations or of the products of one cell lineage with the responsive cells of another lineage. Undoubtedly, some of the interactions reflect the requirements for cell growth under *in vitro* conditions; but others have physiological relevance.

Three major categories of cellular interactions have been defined thus far: the interaction between mononuclear phagocytes and a variety of other haemic cells; the interaction of T lymphocytes with progenitors of granulocytes, mononuclear phagocytes, erythroid cells, and eosinophils; and the cellular interactions of the stromal cells of haematopoietic organs.

Production of phagocytic cells

The principal phagocytic cells are the mature neutrophilic granulocytes and mononuclear phagocytes including monocytes and macrophages. The production of neutrophils is regulated by homeostatic mechanisms that normally maintain the numbers of

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circulating cells within a narrowly defined range. The average neutrophil production rate for man is estimated at 1.6 × 10^9 cells per kg per day. Despite rapid turnover rate and high production volume, the neutrophil production system retains great flexibility in its response to inflammatory stimuli.

The production of neutrophilic granulocytes and mononuclear phagocytes are thought to be closely linked processes; both arise from a common precursor which forms colonies *in vitro* only in the presence of the haematopoietic factor, colony-stimulating activity (CSA)[8,9]. Increasing evidence suggests that CSA is also an important controlling factor *in vivo*[10]. Differentiation along one of the alternative cell pathways may be controlled by the type of CSA or its concentration. Furthermore, there seems to be a family of related glycoproteins with CSA activity, some of which have specificity for different target cells[11].

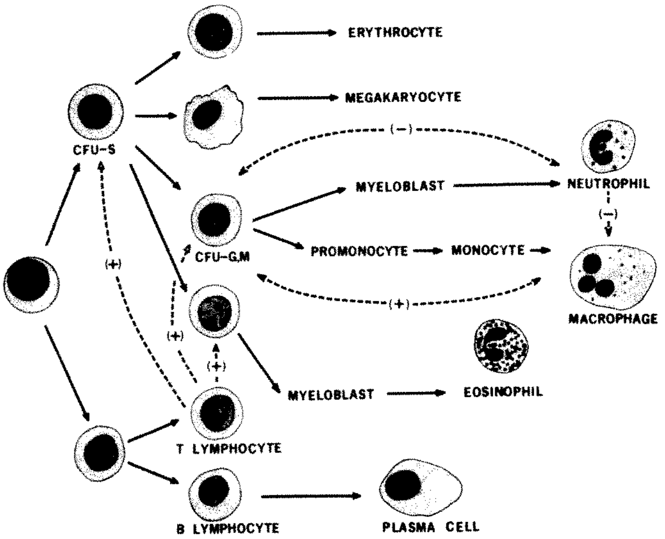

Fig. 1 Granulopoiesis is thought to be regulated by glycoprotein hormones (colony-stimulating activity, CSA) elaborated by monocytes, macrophages, and antigen-stimulated T lymphocytes. CSA acts on the committed myeloid stem cell (CFU-G,M) giving rise to both the neutrophilic granulocyte and the mononuclear phagocyte cell lines. Recent evidence also suggests that products of mature neutrophils may influence the production of both CSA and inhibitors of CFU-G,M by marcophages. If granulocyte chalones exist, they may also inhibit CFU-G,M. Stimulatory interactions are denoted by (+), possible inhibitory interactions by (−).

Only the placenta, certain fetal tissues, mononuclear phagocytes, and stimulated T cells have consistently been shown to serve as a source of CSA for human cells. Mononuclear phagocyte stimulation of granulopoiesis by CSA was the first important haematopoietic cellular interaction to be defined. The following features of this interaction are known: (1) blood monocytes, alveolar, hepatic and peritoneal macrophages all produce CSA[12-14]; (2) malignant monocytes of both mouse and man seem to retain CSA-producing capacity as long as some degree of monocyte differentiation is retained[15,16]; and (3) production of CSA is modulated by exposure of cells to endotoxin, PPD, zymosan, glucan and poly I:C (refs 17, 18). The production of CSA by mononuclear phagocytes has led to a concept of positive regulation of granulopoiesis and monocytopoiesis by products of the responding cells themselves (Fig. 1).

Another important source of CSA is the T lymphocyte. Early observations of granulopoiesis in mice indicated that serum CSA levels were increased in primed animals exposed to antigen. It was subsequently demonstrated *in vitro* that antigen- and mitogen-stimulated T lymphocytes produce CSA, thereby providing an explanation for the *in vivo* phenomenon[19-21].

Monocyte- and lymphocyte-derived CSA are similar and have higher molecular weight components than placental CSA[9]. We have recently established from a patient with a T-cell variant of hairy cell leukaemia (leukaemic reticuloendotheliosis) a line of T cells that produces potent CSA[22]. Production is increased, particularly after exposure of the cells to phytohaemagglutinin (Table 1). This human lymphocyte-derived CSA is sensitive to trypsin and is bound by concanavalin A (Con A).

We conclude that intense antigenic stimulation may stimulate neutrophilic granulopoiesis and monocytopoiesis by an interaction with T lymphocytes mediated by a particular species of CSA. Certain malignant T cells retain this stimulatory capacity.

Negative feedback

We have considered positive controls of the production of phagocytes. Various investigators have also emphasised theoretical reasons why granulopoiesis should be under negative feedback control and have adduced evidence for the existence of granulocyte chalones[23,24]. Chalones are defined as tissue-specific, species-nonspecific products of differentiated cells that selectively inhibit early cells of the same lineage[25]. A putative neutrophilic chalone would be a product of mature neutrophils that would specifically and reversibly inhibit the proliferation of neutrophil precursors but not other dividing cells. Although an inhibitory effect of human granulocyte chalone on human acute leukaemia has recently been reported[26], the existence of granulocyte chalones is controversial. For example, we were unable to find neutrophil-inhibitory products that demonstrate the requisite cellular specificity[27,28].

Another category of possible negative regulators of myeloid stem cells is that of neutrophil-derived factors that inhibit cells producing CSA. Broxmeyer *et al.*[29,30] reported that crude extracts of normal, but not chronic myelocytic leukaemic, neutrophils inhibit the production of CSA by monocytes and macrophages. They therefore postulated that negative feedback from the normal mature neutrophil acted at the level of CSA generation and not at the CFU-G,M level.

Prostaglandins of the E series (PGE_1 and E_2) are potent inhibitors of CFU-G,M proliferation (activity at 10^{-10} M), whereas those of the F series are often stimulatory. The effect of PGE can be overcome with CSA. Human monocytes and mouse macrophages produce both CSA and PGE. High concentrations of macrophages inhibit CFU-G,M growth, and this inhibition is overcome with indomethacin[31,32]. Based on these observations, Kurland and Moore have evolved an elaborate scheme whereby granulopoiesis is modulated by macrophage generation of stimulatory and inhibitory products which in turn is modulated by neutrophil inhibitors and CSA[29-32]. While this scheme remains tentative, the best-defined cellular interaction in granulopoiesis is the positive stimulation of granulopoietic progenitors by products of mononuclear phagocytes and stimulated T lymphocytes (Fig. 1).

Erythropoiesis

The primary control of erythrocyte production is determined by a balance between tissue-oxygen needs and the capacity of the oxygen delivery system, and is mediated by erythropoietin. Recent studies suggest that interactions between erythroid precursors and other cells, particularly the T lymphocyte, may also modulate erythropoiesis.

Two general categories of erythroid precursors have been identified *in vitro*; first, the erythroid colony-forming unit (CFU-E), which is an immediate precursor of the differentiated normoblast and is capable of only three to six divisions; second, the burst-forming unit (BFU-E), which is a more primitive committed erythroid stem cell with potential for many more divisions. The CFU-E is thought to arise from the BFU-E[33]. The CFU-E is sensitive to erythropoietin whereas the BFU-E is relatively insensitive. In the intact animal BFU-E proliferation is influenced by stimuli to marrow regeneration but not by changes in erythropoietin concentration[34]. BFU-E normally circulate in the peripheral blood of man and are found in the null-cell mononuclear leukocyte fraction[35]. When T cells are removed from this fraction, burst formation is diminished; when T cells are restored, so are BFU-E. Other evidence for T-cell–erythropoietic interactions comes from studies of diffusion chambers containing proliferating haematopoietic cells implanted intraperitoneally in mice[36]. Phytohaemagglutinin (PHA)-treated marrow cells or thymocytes added to bone marrow cell suspensions in such chambers greatly stimulate erythroid colony formation (Table 2). Pretreatment with anti-θ serum and complement eliminates the stimulatory effect of the mitogen.

A third line of evidence for a T-cell–erythroid interaction comes from a report that infusion of normal littermate bone marrow depleted of T cells does not cure the genetically determined anaemia of the W/W^v mouse, whereas addition of normal T cells to the marrow results in cure[40]. A fourth line of evidence comes from the observation that medium conditioned by an established T-cell line[22] has erythroid burst-promoting activity. The conditioned medium potentiates the effects of erythropoietin but cannot substitute for the hormone (Table 3). Erythroid burst-promoting activity is reported to be separable from CSA activity in other systems[41].

Several types of evidence therefore suggest that T lymphocytes and their products affect early erythroid progenitors and may thereby interact with erythropoietin in controlling erythropoiesis. Less strong evidence supports a role for mononuclear phagocytes in erythropoiesis. The evidence is essentially morphological and derives from the observation of macrophage-like cells within the bone marrow in the centre of islands of developing erythroid precursors[42]. The effect, if any, of macrophages may be mediated by the prostaglandins which they produce, as PGE_1 causes increased elaboration of erythropoietin by the kidney and has direct effects on stimulating glucosamine uptake and iron incorporation into red-cell progenitors. PGE_1 also stimulates erythroid colony formation *in vitro*. It has recently been suggested[43] that erythropoietin is produced by macrophages in the liver, the major source of extrarenal erythropoietin[44,45]. Alternatively, the generation of erythropoietin in the liver may depend on PGE_1 production by the Kupffer cell, which in turn regulates erythropoietin elaboration by hepatocytes. Circumstantial but not direct evidence therefore links mononuclear phagocytes to erythropoiesis.

Lymphocyte–macrophage interactions

The interactions between mononuclear phagocytes and T and B lymphocytes in antigen processing in the afferent limb of the immune response are so well documented[46] that it is only worth noting their cardinal features. First, they generally require close contact between the mononuclear phagocyte and the responding lymphoid cell. Intact viable mononuclear phagocytes are necessary for optimal response. Surface antigenic identity between the mononuclear phagocytes and the subsequently responding lymphoid cell is generally necessary. Mononuclear phagocytes potentiate the production of biologically active lymphokines, as well as B-lymphocyte antibody responses. Finally, in at least some *in vitro* culture systems, the requirement for mononuclear phagocytes can be replaced by mercaptoethanol and possibly other thiols.

Table 1 Production of CSA by an established human T-cell line

Addition to culture	Duration of culture (d)	Colonies per 10^5 marrow cells
—	2	130 ± 22
—	6	105 ± 20
PHA	2	260 ± 18
PHA	6	272 ± 24

Conditioned medium was prepared from a continuously growing human T-cell line cultured in the presence and absence of PHA and assayed for its ability to stimulate granulocyte–macrophage colonies from non-adherent human bone marrow cells.

The interaction between T lymphocytes and mononuclear phagocytes also operates in the reverse direction: T cells can influence mononuclear phagocyte proliferation, differentiation, and activation[46,47].

Eosinophilopoiesis

Basten *et al.*[48,49] demonstrated a role for large lymphocytes in the mediation of the eosinophilic response to *Trichinella* infection in rats. Irradiated animals developed an eosinophilia in response to parasitic challenge only when reconstituted with lymphocytes as well as bone marrow cells. Marrow alone did not suffice. When lymphocytes from sensitised donors were used together with normal marrow cells, a 'secondary' type of eosinophilic response occurred. In other experiments, neonatal thymectomy significantly reduced the capacity of both mice and rats to mount an eosinophilia in response to antigen[50].

These studies in intact animals have been refined by studies in diffusion chambers implanted in mice and by *in vitro* culture systems[51,52]. Ruscetti *et al.* added medium conditioned by sensitised splenic lymphocytes and *Trichinella spiralis* antigen to marrow cells from *Trichinella*-infected mice. They observed a three- to fourfold increase in eosinophils in liquid culture. The response was antigen specific as neither pokeweed mitogen nor PPD stimulated the release of eosinophil-stimulating factor. Collectively these experiments suggest that thymus-derived or thymus-dependent cells may modulate eosinophilopoiesis in response to antigenic challenge. In addition, T-lymphocyte products may influence eosinophil migration[53]. The granulopoietic and eosinophil responses to the soluble products of sensitised T lymphocytes are probably independent, as studies from *in vitro* culture of marrow from children with congenital neutropenia and other observations[54] support the notion of separate committed stem cells for neutrophils and eosinophils.

Table 2 Stimulation of erythroid colonies from murine bone marrow in diffusion chambers implanted in normal mice

Cells added to chambers	Erythroid colonies per chamber
Bone marrow	1.6 ± 0.8
Bone marrow + PHA	31.9 ± 7.2
Bone marrow + thymocytes	1.7 ± 1.2
Bone marrow + PHA + thymocytes	63.0 ± 22.0

Bone marrow cells (1×10^5) or thymocytes (2×10^4) were incubated for 30 min with or without PHA (0.01 ml per ml) and washed twice. In all cases 100,000 bone marrow cells were implanted in Millipore chambers containing a plasma clot. Erythroid colonies were counted 3 d after peritoneal implantation of chambers in host mice (ref. 36, and E. Niskanen, unpublished observations).

Cellular interactions with pluripotent stem cells

Relatively little is known about the factors influencing proliferation of pluripotent stem cells (CFU-S) or their commitment to a single line of differentiation. Under normal circumstances CFU-S do not actively proliferate. With increased demands for haematopoiesis, as after subtotal irradiation, the CFU-S compartment begins to expand. The signals for proliferation in the intact animal are not known. *In vitro*, PGE_2 has been reported to induce CFU-S to enter the proliferative cell cycle[55]. PGE is one of the biologically active products of the macrophage and, in theory, is a potential mediator of several haematopoietic cell interactions.

Table 3 Erythroid burst-promoting activity of medium from a human T-cell line

Addition to culture	BFU-E per 2×10^5 cells*
None	0
Epo, 3µl per ml	13±2
Epo, 3µl per ml + T-cell medium, 50 µl	42±3

*Light density human peripheral blood leukocytes.

Cerny[56,57] described another possible interaction. He reported that the number of CFU-S increased in spleen cell suspensions treated with phytohaemagglutinin (PHA). The effect was ablated by pretreatment with anti-θ serum and complement. Furthermore, conditioned medium from PHA-treated spleen cells increased the numbers of CFU-S in bone marrow two- to threefold. Pritchard[58] showed that thymocytes augment spleen colony formation when injected with marrow cells. Recent observations suggest that thymectomised mice or nude mice deficient in T cells may also demonstrate abnormalities of haematopoietic stem cell function[59]. If corroborated, these many observations suggest the existence of yet another T-cell interaction with haematopoietic stem cells.

Stromal cell interaction

Another important class of haematopoietic interactions is that between proliferating haematopoietic cells and the stromal cells of blood-forming organs. At present, this class of interactions is the least understood. After transplantation into irradiated syngeneic mice, haematopoietic stem cells proliferate with a doubling time of 20–25 h. The major sites of cellular proliferation are the spleen and the medullary cavity. The rate of stem cell replication and the direction of differentiation are partly determined by factors derived from the organ of lodgment and collectively referred to as the haematopoietic inductive microenvironment (HIM) or the 'stroma'. Some of the factors seem to be under genetic control[60,61].

The murine bone marrow and spleen stroma can support differentiation of pluripotent haematopoietic stem cells along four pathways: erythroid, neutrophilic, eosinophilic, and megakaryocytic[60,61]. Splenic haematopoiesis, however, is prominently erythroid, whereas granulopoiesis is more evident in the bone marrow. The effects of the stromal microenvironment on stem cell differentiation are presumed to be mediated by short-range cellular interactions.

An elegant and clear demonstration of the effect of the HIM on haematopoietic cell differentiation was provided by Wolf and Trentin[60]. They implanted plugs of bone marrow stroma in the spleen and studied the pattern of growth of transplanted haematopoietic stem cells growing across the junction of such implants. On the spleen side, the colonies were predominantly erythroid, and on the marrow side, predominantly granulocytic. Subsequent investigations have suggested that an abnormal HIM for erythroid differentiation is responsible for the genetically determined anaemia of sl/sl^d mice[37–39,61].

There have been few attempts to study organ influences of haematopoiesis *in vitro*. Metcalf found that lymphocytes and thymocytes potentiated colonial bone marrow growth *in vitro*. Subsequently, Chan and Metcalf observed that femoral bone shafts could condition medium to support the growth of marrow colonies[62]. Dexter *et al.*[63–65] have recently developed a liquid culture system in which proliferation of mouse haematopoietic stem cells can be maintained *in vitro* for long periods. In this system, marrow-derived adherent cells established in culture for three weeks are inoculated with freshly isolated bone marrow cells. The latter subsequently proliferate for several months and retain viable CFU-S and CFU-G,M. Their proliferation depends upon the adherent population comprised of phagocytic mononuclear cells, or 'epithelioid' cells, and giant fat-containing cells which seems to provide an *in vitro* microenvironment. Within this adherent layer, extensive cellular interactions occur. Marrow-derived adherent cells from a particular mouse strain can stimulate the growth of haematopoietic cells from genetically incompatible, as well as syngeneic, mice. Dexter and Moore have recently shown functionally abnormal adherent feeder layers derived from sl/sl^d mice[65].

We have also used suspension and agar cultures of mouse bone marrow to study the effects of organ stromal factors on cellular proliferation and differentiation *in vitro*[66]. Bone, spleen, and thymus fragments from irradiated mice were placed in contact with syngeneic bone marrow cells growing in suspension culture. Neutrophilic granulocyte numbers were greatest in the presence of bone or spleen. The stromal microenvironment of, those tissues thus influences haematopoietic cell differentiation as well as proliferation *in vitro*, possibly by means of a CSA-like factor.

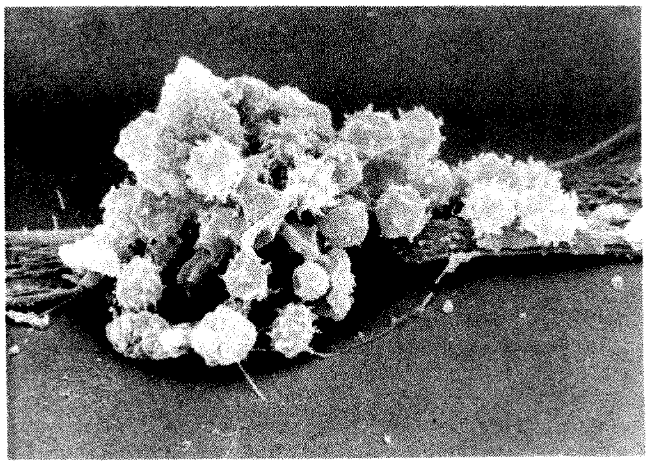

Fig. 2 Scanning electron photomicrograph of cells in long-term culture of human bone marrow. Adherent cells are established in culture before the system is 'recharged' with a fresh inoculum of bone marrow cells. The small round cells proliferate on the surface of larger adherent cells for several weeks and can be assayed in gel-matrix culture as CFU-G,M and CFU-E (photograph courtesy of Dr William Hocking and Shirley Quan of UCLA).

Recently the culture system developed by Dexter *et al.*[63–65] has been applied to the cultivation of human bone marrow. In this system, CFU-G,M and CFU-E are preserved for several weeks. As in the murine system, large stromal cells as well as smaller round proliferating cells are in intimate contact in the growing cultures (Fig. 2). The interactions between these cells which are crucial for proliferation and differentiation are still undefined. Nevertheless, these new culture techniques do provide the means to explore stromal cell interactions.

Leukaemia

In acute myeloid leukaemia, abnormal haematopoiesis results in anaemia, thrombocytopenia, and decreased granulopoiesis. Are the normal haematopoietic cells replaced by leukaemic cells in the blood-forming organs or are they suppressed by humoral factors produced by leukaemic cells? The latter explanation is suggested by cytogenetic studies which reveal that normal haematopoietic clones are present but do not expand until the leukaemic cell mass is reduced by chemotherapy.

Leukaemic cells co-cultivated with normal marrow cells suppress granulopoiesis[67,68]. The basis of this may be in the high molecular weight inhibitor of normal human granulocyte stem cells that we have identified in a previously described myeloid leukaemia cell line[69,70]. This inhibitor also affects mitogen-stimulated human lymphocytes but not erythroid precursors, normal fibroblasts, or various epithelial cell lines (Table 4). It has a molecular weight greater than 100,000, demonstrates species specificity, adsorbs to Millipore filters, and is inactivated by heat at 56 °C after 30 min. Its effect on sensitive stem cells is reversible in a time- and concentration-dependent fashion. It does not seem to affect either the S-phase of the cell cycle or mitotic events. We do not yet know if similar inhibitors are present in clinical leukaemia.

Another consideration is whether leukaemic cells retain responsiveness to normal cellular interactions and haematopoietic hormones. In general, human acute myeloid leukaemic cells in agar gel form small clusters of 4 to 20 cells rather than true colonies of 50 to several hundreds of cells. Nor is there much evidence of cytodifferentiation *in vitro*, although many leukaemias show both enhanced cluster and colony formation in response to CSA. It has been speculated that acute myeloid leukaemia sometimes results from a defect in the haematopoietic environment and its controlling hormones[71]; however, based on the observations cited and the demonstration that leukaemic cells retaining monocytic features can make CSA, it seems unlikely that the primary abnormality in leukaemia resides in the hormonal environment. The fundamental defect seems to be a loss of the ability to differentiate in response to normal hormonal signals and cellular interactions.

Concluding remarks

A variety of cellular interactions control the production of blood cells in haematopoietic organs. These interactions include the involvement of T lymphocytes in granulopoiesis, monocytopoiesis and erythropoiesis; and mononuclear phagocyte interactions in granulopoiesis, in T-lymphocyte differentiation, and perhaps in erythropoiesis. The stromal cells of haematopoietic organs provide a unique microenvironment which influences the development of stem cells.

The rich diversity of haematopoietic cellular interactions may serve as a model for interactions in other tissues and organs comprised of multiple cell types. Blood cell interactions have been elucidated *in vivo* in animal models and *in vitro* in recently developed culture systems applicable to human studies.

For several years cellular interactions affecting haematopoietic cell proliferation and differentiation have been described in terms familiar to cell biologists: for example, the activation of macrophages by soluble products of lymphoid cells is well established[46]. More recently, permanent lines of haematopoietic cells have provided tools for studying induced differentiation of eukaryotic cells at the molecular level[72,73]. It is likely that these model systems will provide the ultimate explanations of the influence of one cell upon the proliferation and differentiation of its neighbour. It is also possible that the techniques used to examine these interactions may have unanticipated benefits for the treatment of human disease. For example, it has recently been reported that mixtures of haematopoietic stem cells cultured for long periods *in vitro* can change their antigenic characteristics and subsequently proliferate in host animals which would ordinarily reject their growth[74]. This observation may have important implications for the biology and technology of human bone marrow transplantation for aplastic anaemia and malignant disease. Currently such transplantation is restricted to donors and recipients possessing haematopoietic cells which are closely similar in surface antigenic structure. The possibility that one could artificially alter that structure is challenging.

Table 4 Inhibition of proliferating cells by medium from a myeloid leukaemia cell line

Target cells	Inhibitor concentration v/v	Inhibition of growth (%)
Human CFU-G,M	1:100	98±2
Human CFU-E	1:10	64±4
	1:100	6±4
Mouse CFU-G,M	1:10	74±6
	1:100	23±5
Human fibroblasts	1:10	2±2
Human epithelioid cells 253J	1:10	0±2
Human T lymphocytes (PHA)	1:10	82±6

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articles

Superluminally expanding radio sources and the radio-quiet QSOs

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We propose a model of apparent superluminal expansion based on straightforward relativistic motion. The model gives a natural explanation of the ratio of radio-quiet to radio-loud quasars.

THE expansion of the double structures of quasars revealed by VLBI[1,2] at apparent separation speeds exceeding $2c$, has posed an intriguing problem; the numerous suggestions that have been made for its solution have been classified and discussed by Blandford *et al.*[3]. Theories of the type they call 'ballistic', in which radio-emitting matter is projected at $v \sim c$ in directions making small angles θ with the direction towards the observer, have been current for a long time, and a version involving spherically symmetrical relativistic expansion was originally devised by Rees[4] to account for the rapid variability of certain powerful radio sources. In a two-component version of the theory, the approaching component has apparent transverse speed $v \sin \theta/(1-(v/c) \cos \theta)$ and apparent power $P_0\{\gamma(1-(v/c)\cos \theta\}^{-(3+\alpha)}$; for the receding component v is replaced by $-v$ (ref. 5) (P_0 is the radio power per Hz in the component's rest frame, and α its spectral index in the sense $P \propto \nu^{-\alpha}$). The maximum apparent transverse speed occurs when $\sin \theta = 1/\gamma$ and is γv; thus an apparent speed of $5c$ calls for γ a little >5.

The traditional difficulties with this type of model are: (1) if their intrinsic powers are at all similar, the receding component has a flux density very much smaller than that of the approaching component; even for modest v/c this ratio is large (see ref. 6) and for $\theta \sim 1/\gamma$ with $\gamma \geq 2$ the receding component should be quite unobservable. This totally disagrees with the reported fairly symmetrical double structure. (2) In a randomly orientated sample of sources, very few have axes sufficiently close to the line of sight to reach large v_{apparent}/c; for example, for $\gamma = 5$ the proportion with $\sin \theta < 1/\gamma$ is 2%. However, superluminal velocities are observed in a large proportion of the quasars that have been observed systematically by VLBI.

Lynden-Bell[7] has shown one way out: he postulates that the ejecta merely excite the surrounding (~stationary) matter to emit radio waves, thus avoiding difficulty (1), and removes (2) by using a large value of Hubble's constant, so that only modest γ's are needed.

Another way is to suppose that the observed VLBI 'doubles' consist of approaching components only. In this scheme the appearance of an expanding double is due to a bright knot in the approaching stream, together with radio emission continually regenerated near the quasar nucleus; a relatively small total energy is enough to provide observable radio emission from a very compact source near the nucleus, but the amount of radio emission there is limited by synchrotron self-absorption, so that we may expect the radio emission close to the nucleus to fluctuate rather less than the energy supply. Difficulty (1) is then irrelevant, and (2) is attributed to a selection effect, sources approaching with high γ and small θ being observed often because of their high apparent radio power. The much larger number of similar sources with large θ are then to be identified with some or all of the radio-quiet quasars, which are optically very similar to quasi-stellar radio sources (QSRS). We have favoured this model for some time, but the stimulus to working out its consequences more seriously came from recent work at Caltech[8-12] which shows that, in six out the eight cases observed, the VLBI structures in fact consist of a (so far) unresolved, self-absorbed component at one end, and a single 'jet' (with a steeper spectrum) emerging from it.

The six sources showing clear core-jet structure are 3C120, 3C147, 3C273, 3C286, 3C345, 3C454.3; the two other sources, CTA102 and 3C380, show no striking asymmetry, but have so far been observed at only one frequency.

Here we define the nature of the model we propose; compute the ratio of total optical QSO numbers to QSRS that it requires; examine the problem of compact central sources associated with large classical doubles; and point out some other observational consequences of the model.

The model

We postulate that the radio-emitting objects are ejected often, or continually, but by no means steadily, along one axis. Thus there is always some radio emission close to the QSO, but all the radio-emitters on the approaching side are moving with similar velocities. (A stationary nuclear component would have flux density independent of orientation, and thus defeat the model through difficulty (2)). We must suppose, however, that much of the light comes from a stationary source, as the line emission does not have large v/c (otherwise blueshifts would occur), and the line: continuum ratio does not vary really drastically from one quasar to another. The model would not be inconsistent with an appreciable fraction of the continuum originating in the relativistic jet in some cases, and indeed such an interpretation would be required if a strong trend for greater optical variability among QSRS than among QSOs as a whole (see ref. 13) is confirmed.

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We need a range of γ extending up to at least 7 (to account for v_{apparent} in 3C345), and down to 1.5 or less to account for the less powerful but relatively common central components of classical double radio sources. Though most of our calculations refer to quasars with only a single value of γ (and also of P_0, α, and optical luminosity) and we concentrate on the distribution in θ, a more thorough treatment will require model-building with distributions of these parameters.

Blandford has pointed out that, if a succession of pairs of components is ejected, and each lasts a given proper time (after which its radio emission fades), then at any instant we observe more of those on the receding side than on the approaching side; thus one Doppler shift factor disappears from Ryle and Longair's formula[5] for the apparent radio power, that is, the exponent becomes $(2+\alpha)$ instead of $(3+\alpha)$. The VLBI observations indicate that we are generally observing several components at once, so that our model will generally correspond more closely to Blandford's picture than to that of Ryle and Longair; hence we adopt an exponent $(2+\alpha)$ in our formulae, and show numerical results corresponding to the strict two-component model by giving α values 1.0 in excess of normal spectral indices.

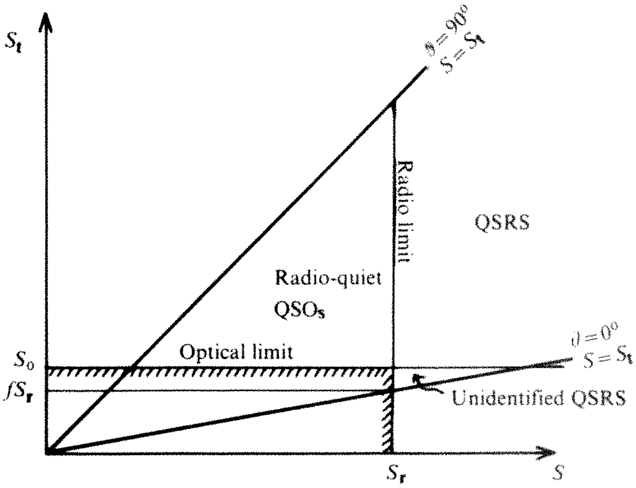


Fig. 1 The S (radio flux density) $-S_t$ (essentially optical flux density) plane. Sources exist between the lines marked $\theta=0^\circ$ and $\theta=90^\circ$.

The number of radio-quiet QSOs

In our model, the approaching half of the radio source gives essentially all its observed flux density S; if it lay transversely in the plane of the sky, it would have given a flux density S_t, where

$$S_t = S(1-(v/c)\cos\theta)^{2+\alpha} \tag{1}$$

(Thus a symmetrical double source with $\theta = 90^\circ$ would have flux density $2S_t$; as in our model the radio emission is very faint for sources with large θ, we shall ignore this fine distinction between S_t and $2S_t$.) We introduce S_t because it depends on distance like S, but is independent of θ, and for our standard quasar we can therefore regard S_t as a measure of the optical flux density.

For our rough estimate, we take the optical differential source count for quasars to be a power law

$$dN = KS_t^{-\beta-1}\,dS_t \tag{2}$$

The density of quasars in the S, S_t plane is then given by

$$\begin{aligned} dN &= KS_t^{-\beta-1}\,dS_t d(\cos\theta) \\ &= \frac{Kc}{v(3+\alpha)} S_t^{-\beta-1+1/(2+\alpha)} S^{-1-1/(2+\alpha)}\,dS_t\,dS \end{aligned} \tag{3}$$

(using equation(1)) in the band between the lines $S_t = S$ and

$$S_t = S(1-v/c)^{2+\alpha} = fS \tag{4}$$

Note that $f \ll 1$ even for modest values of γ: $f \lesssim 0.01$ for $\gamma = 2$ and $f \simeq (2\gamma^2)^{-(2+\alpha)}$ for large γ. If we set optical and radio limits of detection at S_0 and S_r, respectively, such that an appreciable fraction of the sources with $S > S_r$ are optically identified (Fig. 1), then $S_r \gg S_0$ and we find:

Fraction of QSRS with $S > S_r$ that are not optically identified =

$$\begin{aligned} &(1-f^{\beta-1/(2+\alpha)})^{-1}\{1-\beta(2+\alpha)(fS_r/S_0)^{\beta-1/(2+\alpha)} \\ &\qquad + ((2+\alpha)\beta-1)(fS_r/S_0)^{\beta}\} \end{aligned} \tag{5}$$

Fraction of QSO with $S_t > S_0$ which are also QSRS with $S > S_r$ =

$$\begin{aligned} &\frac{c}{v}\left\{\frac{\beta(2+\alpha)}{\beta(2+\alpha)-1}(S_0/S_r)^{1/(2+\alpha)} - (1-v/c) - \frac{1}{\beta(2+\alpha)-1}(S_0/S_r)^{\beta}\right\} \\ &\simeq \frac{1}{2\gamma^2}\left\{\frac{\beta(2+\alpha)}{\beta(2+\alpha)-1}(S_0/fS_r)^{1/(2+\alpha)} - 1\right\} \end{aligned} \tag{6}$$

We can safely set the first factor in equation (5) to unity; eliminating the quantity (fS_r/S_0) between equations (5) and (6) then lets us present the relationship between the fraction of unidentified QSRS and the fraction of QSRS in an optically selected sample in the simple form shown in Fig. 2.

Evidently, if most of the QSRS are optically identified (which is certainly true of flat-spectrum quasars), the ratio of total QSOs to QSRS is several $\gamma^2:1$; if all are identified $(S_0 < fS_r)$ the ratio exeeds $2\gamma^2\{\beta(2+\alpha)-1\}$ that is it is $>4\gamma^2$ for $\beta = 1.5$, $\alpha = 0$. The numerical factor does not depend critically on the proportion of unidentified quasars, or on β or α; thus if γ is typically 5, we expect about 1% of an optically selected sample to be radio sources. Furthermore, we expect the proportion to rise very slowly if we look with more sensitive radio telescopes; equation (6) shows that the proportion of QSRS rises roughly like $S_r^{-1/(2+\alpha)}$. This feature, too, is qualitatively entirely in agreement with observational experience.

The distribution of apparent velocities

We can gain insight into the apparent velocity distribution in a radio-selected sample by working in the approximation $\gamma \gg 1$, $\theta \ll 1$. To this approximation, the proportion of sources with radio flux density $S > S_r$ having $v_{\text{apparent}}/c > \gamma\sin\chi$ is

$$(\cos\tfrac{1}{2}\chi)^{2\beta(2+\alpha)-2} - (\sin\tfrac{1}{2}\chi)^{2\beta(2+\alpha)-2} \tag{7}$$

With $\beta = 1.5$, $\alpha = 0$ ($\beta = 1.8$, $\alpha = 1.5$), 87% (69%) of sources have $v_{\text{apparent}}/c > 0.5\gamma$ and 60% (31%) have $v_{\text{apparent}}/c > 0.8\gamma$. Thus apparent velocities comparable with γc are indeed expected to be very common in a radio-selected sample. (The exact figures are 82% (62%) and 39% (16%) at $\gamma = 2$, so the approximation is a fair guide even for small γ.)

Central components of classical double sources

In classical double radio sources, where the central component generally contributes little to the total flux density (especially at low frequencies), we should have a sample with random orientation of axes. However, a substantial fraction of such sources have central components whose properties seem to be qualitatively similar to compact sources without extended structure, and which are roughly aligned with the large-scale double in the few cases where VLBI measurements exist. No direct measurements of apparent expansion velocities exist yet. (We must exclude sources with extended structure on one side only, such as 3C273, also 3C345 and 3C454.3 (ref. 14) as in these, the extended structure might have substantial v/c). However, a few central components are known to be radio variables.

Clearly, very few of these central components can be sources with $\gamma \gtrsim 5$, as we have seen that for each such source there must be several γ^2 'radio-quiet sources'—here, doubles without observable central components—whereas it is observed that

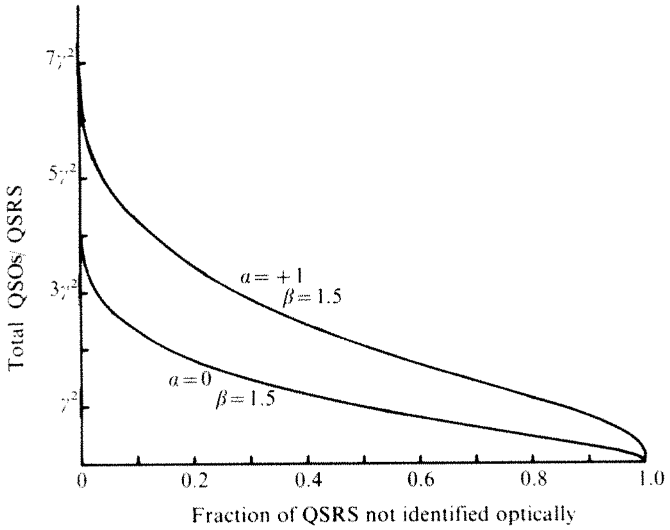


Fig. 2 The ratio of total quasars (radio quiet and QSRS) to QSRS alone, above given radio and optical limits, as a function of the fraction of unidentified QSRS in the radio-limited sample. The differential optical source count is assumed to be a power law with index $-(\beta + 1)$; the line $\alpha = 0$ represents flat-spectrum sources in mean, while the $\alpha = +1$ line represents either steep-spectrum sources or a strict 2-component model for flat-spectrum sources (see text).

~30% of 3C radio doubles (and more than half of the 3C quasars with extended double structure) have central components. We now make this argument a little more quantitative. For sources of identical γ, S_t and α, the distribution of apparent intensities due to their random orientations is given by

$$dN \propto d(\cos\theta) = \text{constant} \times S^{-1-1/(2+\alpha)}\, dS,\ S_t < S < S_t/f \tag{8}$$

and zero outside these limits. To estimate the largest γ we can allow as typical for central components, we compare equation (8) with the narrowest distribution we can find for S relative to some orientation-independent quantity, which is that of the ratio R of S to the flux density of the extended double structure. We use the complete sample of 166 3C sources selected at 178 MHz (listed in ref. 15). From it, we select those sources that: first, have large-scale structure on both sides of the optical position; second, are well enough resolved to yield an estimate or a useful upper limit for the flux density of the central component at 5 GHz (in some cases of doubtful interpretation, 15 GHz maps with 0.67" resolution have also been inspected); third, are identified with quasars.

We use only quasars because central components are commoner in them than in sources as a whole, so that they lead to a severer restriction on γ. (We should also exclude sources whose 178 MHz flux in extended structure alone would have fallen below the $S_r = 10$ Jy limit of the sample, but found no likely cases that had not already been excluded on other grounds.) The result (see Fig. 3) shows that a range of 64 : 1 in the ratio R is compatible with the observations; this corresponds to $\gamma = 2$. Even $\gamma = 2.5$ would be hard to reconcile with the observations: the total number of sources would then have to be six times the number in the top three octaves, that is, 36 instead of the 22 on our list. Of course, the true distribution is likely to contain a range of γ, extending from values close to 1 to $\gamma = 2.5$ and beyond; such a range of γ, together with a scatter in other instrinsic properties, would give a more bell-shaped distribution than equation (8).

We emphasise that there is no need to suppose that the central components of classical doubles are drawn from a population with a quite different γ distribution from that of the compact quasars; information available would allow us to ascribe the apparent difference solely to selection effects, for the central components by themselves would make very little difference to the counts of radio sources, or of quasars.

A more serious matter is the variability of central components. The observational evidence is still very sparse, and the most striking case we know is that of 3C111 at $z = 0.049$, whose central component has been known to vary by 2 Jy in 0.4 yr (R. G. Hine, personal communication). This rate of variation would be compatible with conventional electron synchrotron emission for a component speed corresponding to $\gamma \sim 2$, or rather less if the component is itself expanding with mildly relativistic speed.

The radio source counts at high frequencies

It is well known that the radio source counts at high frequencies (2.7 and 5 GHz) deviate much less from a power law with $\beta = 1.5$ than do the source counts at 408 MHz and lower frequencies (see ref. 16). There are two ways in which this fact could be related to the present model.

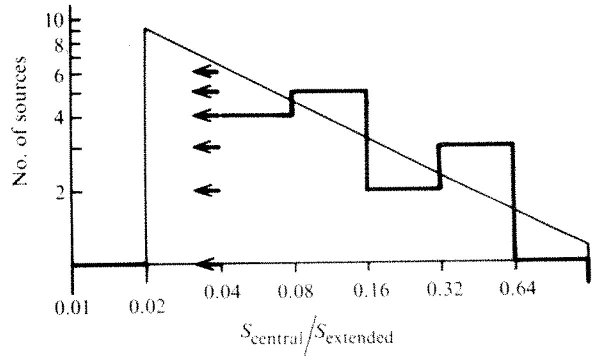
First, the distribution of S with orientation (equation (8)) introduces a broad scatter in apparent radio luminosity in addition to the intrinsic radio luminosity function. This might be expected to flatten out the hump in the graph of normalised source counts versus S (Fig. 1 of ref. 16) at high frequencies, where the source counts are dominated by flat-spectrum quasars, but not at low frequencies, where the source counts are dominated by classical doubles and orientation effects are therefore unimportant. In fact, this effect is far too small to account for the observed difference between low and high frequency counts, because the distribution in equation (8) is so much less steep than the source counts. Numerical convolution of the 408-MHz source counts (from ref. 16) with distributions given by equation (8) flattens the graph of normalised source counts slightly though noticeably at low flux densities, and changes its slope very little indeed at high flux densities.

Second, it is also well known that low-powered radio sources evolve far less strongly than high-powered radio sources. In the present model, the flat-spectrum quasars are sources of low intrinsic radio luminosity, promoted to high flux density by high speed and favourable orientation. Thus the present model may provide a link between the relatively slow evolution of flat-spectrum quasars and the relatively slow evolution of weak radio sources.

The curvature of the VLBI structure

Readhead *et al.*[10] have considered the VLBI structure of radio sources which have extended components on either side of the optical object, and of sources in which the extended structure, if any, is one-sided and a dominant compact (<0.1") radio

Fig. 3 Histogram of the numbers of classical double quasars with various ratios of central component flux density to extended component flux density (note log scales). The thin line is the predicted distribution for the model sources with flat spectra ($\alpha = 0$) expanding at $\gamma = 2$, fitted to the histogram; larger γ would require a larger range along the abscissa, and hence a greater number of sources without observable central components.



component is coincident with the optical object. They call these type S and type C objects, respectively, and find that, in those type S objects that have a sufficiently bright central radio component to be studied by VLBI, this structure is well aligned with the outer components (five cases). Conversely, in type C objects the compact component is generally curved (four cases). If our model is correct, the fact that curvature is seen at all implies that the opening angle of the jet is considerably less than $1/\gamma$. The curvature observed, projected on to the plane of the sky, will be the instrinsic curvature of the jet magnified by a factor cosec θ, that is a factor $\gtrsim \gamma$ according to our model. Thus our model leads us to expect much greater curvature in type C sources (large γ, small θ) than in type S sources (small γ, random θ). The curvature observed in type C sources ranges from 20° to 45°.

Conclusions

We believe that the model suggested here is consistent with the known facts about quasars. The model lacks ingenuity, and we regard that as one of its merits. The model is a fragile one; the clearest and cleanest evidence against it would be the detection of large superluminal apparent expansion velocities in the central components of even a few quasars with extended double structure. As a preliminary to looking for such evidence we hope to encourage more extensive monitoring of flux density variations in such central components.

We note that the high speeds of approach in the present model increase the limit on apparent surface brightness imposed by the onset of inverse Compton scattering; thus it is rather easier to account for rapid variability below 1 GHz[17,18] than it would be in models in which the radio emitters are static.

Much more detailed model-building will be required to find out whether the model is consistent with all aspects of quasar statistics, such as their distribution in redshift and in optical luminosity. We have also treated the radio components as being ejected into an infinitesimal cone at a single value of θ; ejection into a finite range of θ could have substantial effects on the statistics. The different typical γs required for compact quasars and quasars with extended double structure seem at first to be a very contrived feature of the model, but it seems to us that this difference can arise largely or entirely as a result of the different methods of selection. We are much less happy about the need to attribute relativistic speeds to the single-ended extended structures in 3C273, 3C345, and 3C454.3, though we are consoled by the facts that (1) these extended structures are in all three cases on the same side of the most compact components as is the jet, and (2) two such structures (in 3C273 and in 3C274 (M87)) are also distinguished from other extended radio structures by their strong optical continuum emission.

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On the origin of the universal dielectric response in condensed matter

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The observed dielectric response of most materials is seldom in accord with Debye behaviour, but has been found experimentally to exhibit a remarkable 'universality.' It is proposed that this universality is a consequence of some ubiquitous correlated states which exhibit an infrared (IR) divergent-like response to induced transitions of the polarising species.

IN a recent detailed survey[1-4] of the dielectric response of a wide range of solids, it was concluded that the time or frequency response departs strongly from the Debye response and that it falls into a remarkably common or 'universal' pattern in which the frequency dependence of the dielectric loss follows the empirical law

$$\chi''(\omega) \propto \omega^{n-1} \quad \text{with} \quad 0<n<1 \tag{1}$$

extending over several decades of frequency from the lowest audio and sub-audio range to $\omega/2\pi \simeq 10^9$ Hz, in a remarkably wide range of different materials. The corresponding time-domain response of the depolarisation current is given by the Fourier transform of equation (1) and has been known for many years as the Curie–von Schweidler law:

$$i(t) \propto t^{-n} \tag{2}$$

The universal response applies to permanent dipoles and to hopping charge carriers of electronic, polaronic and ionic nature. It is valid in solids with covalent, ionic and molecular bonds, in single crystals, polycrystalline and amorphous structures, and hence is apparently independent of the particulars of the material. At higher frequencies, in excess of 10^9 Hz, quantum effects involving lattice mode excitations and/or electronic excitations become prominent but these will not interest us in the present context.

The various types of dielectric response are summarised[4] in Fig. 1. We note the virtual absence of the pure Debye behaviour. In dipolar materials the universal response, equation (1), is followed at low frequencies by a loss peak of either the α or the β type, while in systems dominated by hopping charge carriers

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another universal response with very small values of n, typically between 0.1 and 0.3, follows at low frequencies[5].

The totality of these experimentally ascertainable facts is not compatible with any of the numerous theories that have been proposed in the last half-century to explain the behaviour of this or that particular class of materials and the need to invoke many-body interactions to account for the generality of equation (1) has been suggested[2-4]. The possible connection of this behaviour with infrared divergent response has also been conjectured (K. L. Ngai, unpublished).

This state of affairs has motivated us to seek a more fundamental understanding of the universal behaviour in terms of physically simple and 'elementary' properties. We will present here several elementary principles which when combined will enable a derivation of the universal law shown in equation (1) to be made regardless of the physical, chemical and geometrical properties of the solids, and also regardless of the nature of the electrically active species responsible for polarisation, whether dipoles, electrons or ions. We will also discuss several examples of low energy excitations expected in various systems that satisfy these elementary principles. We shall derive the universal response (1) and briefly mention the possible presence of a loss peak at lower frequencies.

IR divergence and the universal law

IR divergence phenomena, although not commonly observed in physics, have been seen in several instances, notably in quantum electrodynamics as bremsstrahlung[6], and in solid state physics[7,8] as the peculiar shape of X-ray absorption edges of metals[9]. These examples are not exhaustive but the subjects they cover demonstrate that IR divergence is not uncommon.

The features common to systems exhibiting the IR divergence are: (1) the sudden application of a potential, or a sudden change of the potential or the hamiltonian; and (2) the availability of low-energy excitations of the system and its response to the sudden potential change dominated by the emissions of these low-energy excitations.

In the time domain, the phenomenon is the transient response[10-12] of the system to that abrupt change of potential. IR divergence occurs whenever the suddenly switched on potential V excites some low energy excitations, with a density of states $N(E)$ for excitation energy E, which is such that $V^2(E)N(E) \propto E$. In this instance there is an increasingly high probability of exciting decreasingly small energy excitations and this causes a power-law divergence of the response in the frequency domain, equation (1), or its time-domain equivalent, equation (2). In the X-ray edge problem in metals an absorbed X-ray photon suddenly switches on a hole–core potential V for the conduction electrons. The low-energy excitations here are the electron–hole pairs.

We shall propose later that within a broad classification of dielectrics some ubiquitous states exist that, for convenience, we shall refer to as correlated states. Low energy excitation of the correlated states with excitation energy E consists of removing an 'occupied' state to an 'unoccupied' state and is the analogue of the electron–hole pair excitation in the X-ray edge problem. Due to induced transitions of the charged particles or dipoles responsible for the polarisation of the dielectric, a potential V is turned on acting on these correlated states. Hence the response of the dielectric involves emission of correlated state excitations. We shall argue in a specific example given that the low energy excitations of these correlated states have a density of states $N(E) \propto E$, and the potential change V has little or no E dependence. Furthermore, V may be taken as suddenly applied as the polarising species of the dielectrics should undergo quantum transitions, including changes in their positions/orientations, between preferred states in an abrupt manner by hopping or jumping movements[1-4] such that the time $1/\nu$ taken by the actual transition is negligible in comparison with both (1) the time spent on average in the respective preferred states, and (2) the time characteristic of the low energy excitation of the correlated states. Condition (1) is invariably satisfied in solid dielectrics. That condition (2) is also satisfied will become clearer after we have considered the nature of the correlated states. It then follows that the requirements for an IR divergent dielectric response of the correlated states are satisfied. The mean number p of correlated state excitations is then $p = b\int_0^{E_c} V^2 N(E)dE/E^2$, which diverges logarithmically, where E_c is the upper 'cut-off' at the correlated state excitation energy.

It is interesting that the IR divergence problem when considered in the time domain as a transient response problem[10-12] does lead to the time decay of the response function for large times as t^{-n}. The derivation of the complete dipolar loss peak response will be discussed elsewhere, meanwhile we discuss the correlated states in a broad classification of dielectrics.

Correlated states

We have connected the universal law, equation (1), to an IR divergent response of correlated states. For this interpretation to follow, such states must be prevalent in dielectrics and have characteristic response times long in comparison to the switching of the hamiltonian. Below, we discuss several examples of such correlated states which can reasonably be expected to be present in many dielectrics. Note, however, that although these examples are quite general, they are by no means exhaustive. Furthermore, the first class of correlated states which we discuss (those related to spin density fluctuations) is not the most general as the majority of dielectrics are diamagnetic. We have chosen this class for initial consideration because the resultant analysis facilitates the treatment of the more important classes of correlated states considered subsequently.

(1) Dielectrics with localised spins: new concepts and ideas on low-energy excitations in spin glasses (and real glasses) have been recently introduced by Anderson *et al.*[13], Phillips[14] and by Anderson[15]. They propose the existence of a statistical distribution of localised tunnelling levels and/or modes. In a spin glass, spins are considered as classical dynamical quantities with a potential energy surface that is a function of the simultaneously specified orientations of all the spins; local minima in the energy correspond to metastable states of the spin glass associated with different spin configurations. A tunnelling mode (TM) in a spin glass[13,15] is defined in spin configurational space as two local minima of energy difference ΔE separated by a quantum-mechanical energy barrier. Tunnelling between one local minimum and another, if it occurs, involves the rearrangement of several spins. The 'low' temperature linear specific heat observed in spin glasses comes from TMs with energy barriers that are sufficiently great so that resonant tunnelling of spins (atoms) between the local minima does not occur, but small enough so that tunnelling between the two levels can take place during the time span of the specific heat measurement.

Let us examine the transient response of these spin TMs to a sudden potential change, V, caused by fast quantum transitions of some charged species. Tunnelling modes whose alternate states are separated by more than $\sim \hbar\omega_0$, with $\omega_0 = 10$ GHz, can be eliminated at the outset for consideration of IR divergent response as our interest is in the low frequency dielectric behaviour where ω is smaller or much smaller than 10 GHz. Also, we need to consider only the very low energy TMs with quantum-mechanical energy barriers large enough so that the characteristic response time of the TM to V is long in comparison with the jump time of the polarisable species. This class of TMs should exist. Anderson[15] pointed out that as the configurations of the spins (atoms) is random, there must be very many locations (of order N; the number of spins or sets of spins), where there are two possible configurations of very similar energies E_1 and E_2. If E_1 and E_2 are independent random, variables, then the probability $P(\Delta E)$ of finding $\Delta E = |E_2 - E_1|$ is finite as $\Delta E \rightarrow 0$. But physically this is not true because it is possible to tunnel between the two alternate levels with a tunnelling matrix element T_{12} even though this is small for the TMs under consideration. The energy level separation will be at least $\Delta E \geqslant |T_{12}|$; the off-diagonal matrix element between the alternate levels. For the low frequency dielectric response (that

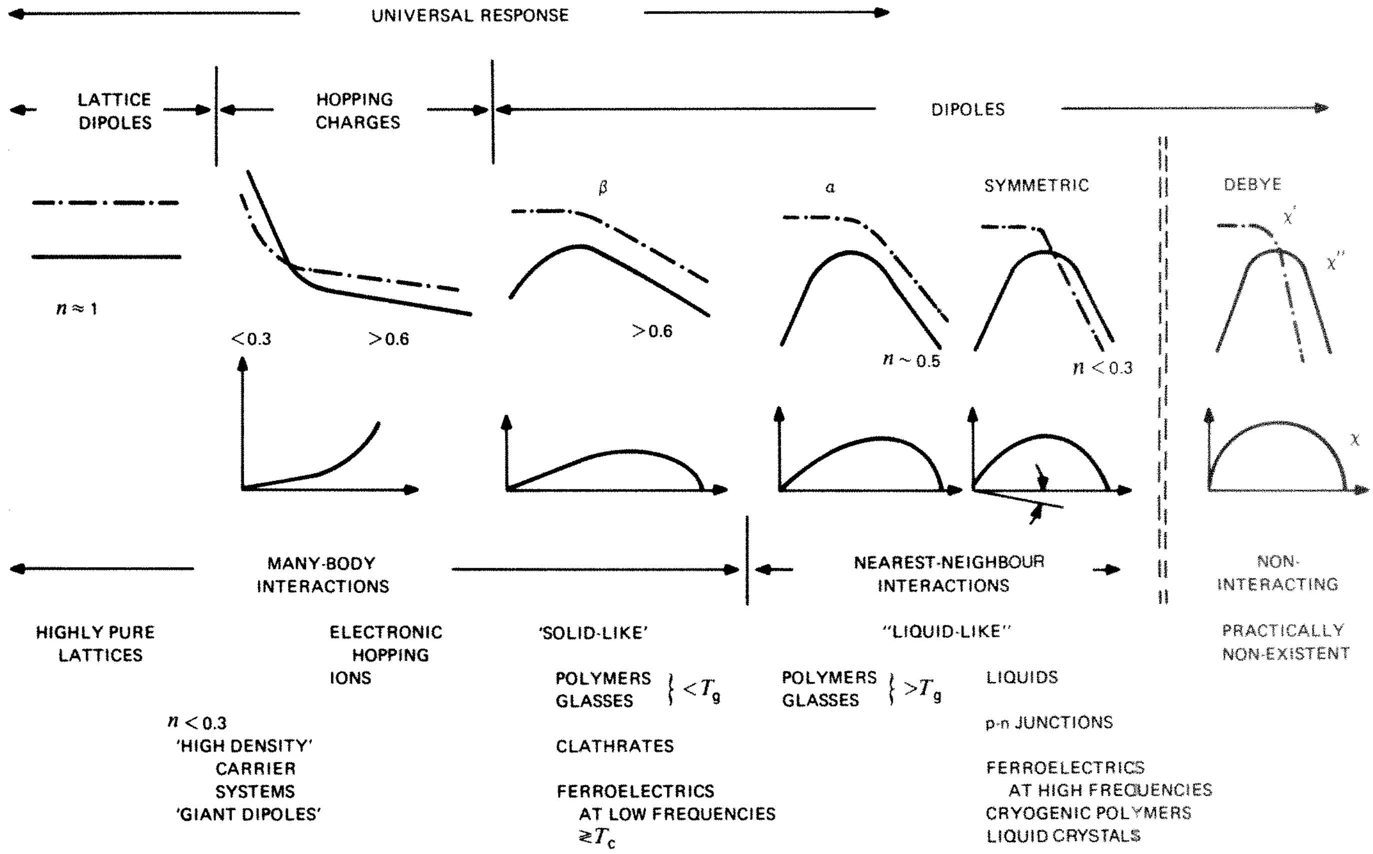

Fig. 1 A representation of the various observed types of dielectric response in the entire range of solids. The upper set of diagrams represent the shapes of the logarithmic plots of $\chi'(\omega)$ (chain–dotted lines) and $\chi''(\omega)$ (solid lines), ranging from the ideal Debye through the α and β peaks and on to the universal dependence for charged carrier systems. The limiting forms of behaviour are represented by the strong low-frequency dispersion with small values of n and by the limiting case of frequency-independent 'lattice response' with $n \approx 1$. The lower set of diagrams represents the corresponding complex χ plots. The various types of materials obeying the respective types are shown and the presumed polarisation mechanisms are indicated. For further details and source references see ref. 4.

is, < 10 GHz) we are particularly interested in the $\Delta E = |T_{12}| \rightarrow 0$ limit. It has been argued by Anderson[15], that T_{12} being a complex matrix element acts like the x and y components of the random field, as it has real and imaginary parts. The probability that the mode energy ΔE lies in the interval $|T|$ and $|T| + d|T|$ is then proportional to $|T|d|T|$. Thus, the density of states of very low energy, tunnelling modes, $N(\Delta E)$, is proportional to ΔE. Now the sudden potential change that induces transitions between the two alternate levels should on the whole not depend on ΔE. Thus, within this class of tunnelling modes, the condition for IR divergence $|V_{12}|^2 N(\Delta E) = n\Delta E$ is satisfied and the universal law follows for spin-glasses. Note that here, and henceforth, (unless otherwise stated) the two states of the TMs are considered thermodynamically accessible to each other. The possible role of thermodynamically inaccessible correlated states in contributing to the divergent dielectric response will be pointed out later. Also note that quite generally systems describable, for example, by a random Hubbard model would be expected to exhibit magnetic properties describable in terms of spin glass states with an example being perhaps impurity bands in crystalline semiconductors.

(2) Dielectrics with atom–atom or molecule–molecule or ion–ion or dipole–dipole interactions: spin–spin interaction models can often be transcribed to other physical models with non-spin interactions[17]. Well known examples include the Ising model equivalence to a lattice gas and to a binary alloy. A lattice gas is a collection of atoms (molecules) whose positions can take on only discrete values which form a lattice. Each lattice site can be occupied by at most one atom. In general the potential energy of the system of atoms corresponds to a gas in which the atoms are located only on lattice sites and interact through a two-body potential $v(|\mathbf{r}_i - \mathbf{r}_j|)$. The correspondence between the lattice gas and the Ising model is then practically immediate[18].

Consider dielectrics were atom–atom, molecule–molecule or ion–ion interactions are important. In the 'lattice' gas and/or binary alloy modelling of dielectrics with random interactions, the essential equivalence to the spin glass Ising model implies that a dielectric state corresponding to the spin glass state exists. Such dielectrics will have, in analogy to spin glasses, very low energy tunnelling modes which correspond to the change of the atomic (molecular or ionic) occupancy of several sites in getting from one energy minimum to the other. This class of tunnelling modes again should satisfy the criterion for IR divergence and hence yields the universal law. The lattice gas and binary alloy model should be good representations of many dielectrics including the class of solid state ionic conductors[19,20] or solid electrolytes such as AgI, CaF and Na β-alumina. In fact, ionic conductivity for these solids has been calculated in the lattice gas model[21]. In the case of Na β-alumina, there is the repulsive interaction among the diffusing sodium ions and also the attractive interactions between the ions and their randomly distributed, compensating defects. These properties imply a lattice gas with random interactions. There is ample experimental evidence[22,23] for the existence of tunnelling modes in alkali β-alumina as well as Ag β-alumina. In particular there is an excess low temperature specific heat[23,24] contribution just as in the case of the spin glasses.

(3) Dielectrics with electron self-trapping states: the concept of local electron self-trapping largely arises from the observation that by and large if a particular electronic state is singly occupied the atom or atoms principally associated with this state will adjust their positions in such a way as to lower the energy level of this state relative to its value when unoccupied. A traditional example is provided by those ionic solids where strong local coulomb interactions between the electron and lattice constituents induces a local lattice distortion leading possibly to

the self-trapping of the electron and the formation of a small polaron. This tendency is not simply restricted to ionic materials but is much more general. Well known examples lying outside the usual small polaron mechanism are provided by the reconstruction of semiconductor surfaces[25,26] and electron pairing states in amorphous glasses[27,28]. The origin of the self-trapping in these instances is essentially a dehybridisation energy[25,26,29,30] arising from concepts inherent in covalency and stereochemistry. The electron–lattice interaction may be strong enough to make it energetically much more favourable to self-trap electrons in pairs rather than singly and such systems have been described by Anderson[27] through a negative U Hubbard model. Let us restrict our attention for the moment to the negative U electron pairing states which should be the physically most important due to the prevalence of diamagnetic systems in nature. in this instance several subgroups of possible correlated states can be identified with properties leading to the desired behaviour in χ'' where, in each case, the response time of these states is expected to be acceptably slow due to the lattice coordinates involved.

Consider first those systems where there are a continuous density of pairing states, the number of which exceeds the number of available electrons. In these instances one can envision very low energy TMs corresponding to different arrangements of the electrons over the pairing centres. By direct analogy with the spin glass TMs these electron-pair TMs should exhibit the desired properties; that is $N(\Delta E)\propto\Delta E$, $V(E)=$ constant.

Another not completely orthogonal subgroup of bipolaron derived TMs that could contribute to the ω^{n-1} behaviour of χ'' is realised when the system is such that bipolaron states are essentially degenerate with, for example, non-distortive extended states. Furthermore, in those instances where the coulomb repulsion dominates, heavily favouring single self-trapping of the electron, various subgroups of correlated states can be identified which are completely analogous to the bipolaron ones outlined above. Intermediate subgroups can also be defined where, for example, one envisages very low energy excitations which entail disassociation of a bipolaron into two singly self-trapped electrons or vice versa.

Consider now an example of a thermodynamically inaccessible correlated state which could produce the desired functional dependence in χ''. For this purpose assume a smooth density of electron pairing states in the neighbourhood of the 'Fermi level' and suppose that a field induced hop of the polarisable species introduces a coupling between these states which is largely independent of energy. In this instance one will expect an ω^{n-1} dependence in χ'' from very low energy excitations that involve the transfer of an electron pair from an occupied to initially unoccupied pairing centre. This behaviour, however, will be completely smeared out at experimental temperatures unless we suppose that the energy barriers between pairing states is sufficient to prevent thermally assisted tunnelling. Thus the system at finite temperature is presumed locked into a metastable state and this could happen for large enough negative U. This case is complementary to our previous examples where it is supposed that each TM has equal probability of being in either of its two states. Note that the possibility of low lying excitations which are thermodynamically inaccessible over at least the time of a specific heat measurement has already been pointed out[15] and in amorphous materials metastable states may persist almost indefinitely. Furthermore in this metastable regime the response of the system should depend on its history.

Although in this section we have considered primarily amorphous systems where one expects an appreciable number of weaker/stronger bonds, lone pairs, and so on, to be present giving rise to the correlated states, it is also reasonable to expect that such low-lying excitations occur and are important in more nearly crystalline solids where, for example, the remaining pairing centres in these materials could partially pin the Fermi level in their vicinity. Another relevant point is the probable presence of an appreciable density of correlated states at various interfaces such as oxide–semiconductor[29], and metal–semiconductor[30]. Thus, although interface or contact effects are usually ignored, we expect that such systems should also exhibit a dielectric loss obeying the 'universal law' and a systematic study of the details could provide a powerful probe of the interfacial structure, so important in device physics.

Low frequency IR divergent dielectric response

Having argued that dielectrics with diverse interaction types should have invariably some very low frequency excitations that contribute a time dependence of the form t^{-n} at large t to some correlation function, we examine further the properties of the dielectric response function. Here we focus on the behaviour of $\chi''(\omega)$ starting several decades above any associated loss peak that may exist, since this is the region where the universal ω^{n-1} dependence is found (see Fig. 1). In doing this we do not exclude those dielectrics which have associated with them the presence of charge carriers of an electronic or an ionic nature. In this regime the dielectric loss is simply related to the probability of exciting the correlated states and is given by $\chi''(\omega)\propto \int_{-\infty}^{+\infty} \mathrm{d}t\, \mathrm{e}^{i\omega t} e^{-\phi(t)}$ where $\phi(t)$ describes the time response of the correlated states due to the sudden potential, V. The function $\phi(t)$ has been obtained many times[7,8] and in our notation takes the form $\phi(t)\equiv\int_0^{E_c} V^2N(E)\,(1-e^{-iEt})\,(dE/E^2)$. The existence of some class of correlated states in the dielectric with $V^2N(E)\equiv nE$ produces an IR divergence in the number of low energy correlated states excited and for large E_ct, $\phi(t)$ can be approximated by $n\ln(iE_ct)$. Putting this result back into the expression for χ'' leads immediately to the universal law $\chi''(\omega)\propto\omega^{(n-1)}$. Note that the loss peak observed in dipolar solids before the universal response can be accommodated as well within the present framework and will be discussed elsewhere. In addition, dielectrics with hopping electronic or ionic charge carriers would be expected to show a strong low-frequency dispersion, in accordance with experimental evidence.

Conclusions

We have arrived at a fundamental mechanism for the universal dependence of the dielectric loss in nearly all dielectrics at frequencies above any loss peak that may be present and the mechanism is operative independent of the physical structure and chemical bonding in the materials. We emphasise the importance here of not only the identification of the Curie–von Schweidler law as an IR divergence phenomenon but also the subtle task of finding the correlated state excitations that are responsible for it.

The entire empirically determined range of values of the exponent, $0<n<1$, can be accounted for in the present theory depending on the values of the exciting potential V arising from the transitions of the charged species and of the density of states of correlated state excitations. The appearance of the limiting form of the Debye response may be expected in situations where V or $N(E)$ are negligible. Our approach then produces a general scheme for classification of dielectrics based on the type of dominant correlations and correlated states they render. In particular, a discussion of correlated states associated with electron–electron, electron–lattice, ion–ion correlations as well as various subgrouping have been given. There may still be other types of correlations leading to correlated states with the same properties. In any event, dielectric loss should provde a powerful probe into details of the correlated states of the type we have considered here. We acknowledge financial support from the NSF and SRC.

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Correlation of the Eemian (interglacial) Stage and the deep-sea oxygen-isotope stratigraphy

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A complete interglacial sequence in coastal marine sediments in western Norway is correlated with the Eemian Stage by means of pollen stratigraphy, and with deep-sea cores by means of marine fossils. The Eemian is correlated with isotope stage 5e.

THE correlation of deep-sea and continental records is one of the most important problems in Quaternary stratigraphy. Because nearly all cores from the deep-sea floor can be correlated using the $^{18}O/^{16}O$ record[1,2], oxygen-isotope stratigraphy is also the most valuable method of correlation with the continents. The general principles in the correlation of glacial/interglacial periods with oxygen-isotope stages are known[1]. However, the correlation of real stratigraphical units defined on land, and the oxygen-isotope stages remains problematic beyond the range of ^{14}C dating.

Here we correlate the Eemian Stage of North-west Europe with the oxygen-isotope stage 5e as follows: a complete interglacial sequence in coastal marine sediments in western Norway can be correlated with the Eemian by means of its pollen stratigraphy, and the marine fauna of the same section can be correlated with the biostratigraphy of cores from the Norwegian Sea[3], in which the oxygen-isotope stratigraphy has also been established[4,5].

Shakleton[6] first argued for the correlation of 5e with the Eemian, and his conclusion has been accepted by most scientists. Confirmation has, however, been lacking, and discussion of alternative correlations has continued. Off the Pacific coast of North America the correlation of 5e with the forest vegetation period of the last interglacial has been demonstrated[7].

Definition and stratigraphical position of the Eemian

The Eem interglacial is now generally accepted as a chronostratigraphical unit with stage rank: the Eemian Stage[8-10]. The type locality is along the river Eem, near Amersfoort, Netherlands[11]. The lower boundary is defined as where a subarctic park landscape at the type locality is replaced with a closed forest and the upper boundary is placed where the forest at the type locality is replaced by a more open vegetation.

The Eemian in North-west Europe is clearly recognisable by its typical pollen stratigraphy[12,13]. This approach could create confusion if the vegetational development during two interglacials were very similar. However, this does not seem to be a serious problem for the Eemian.

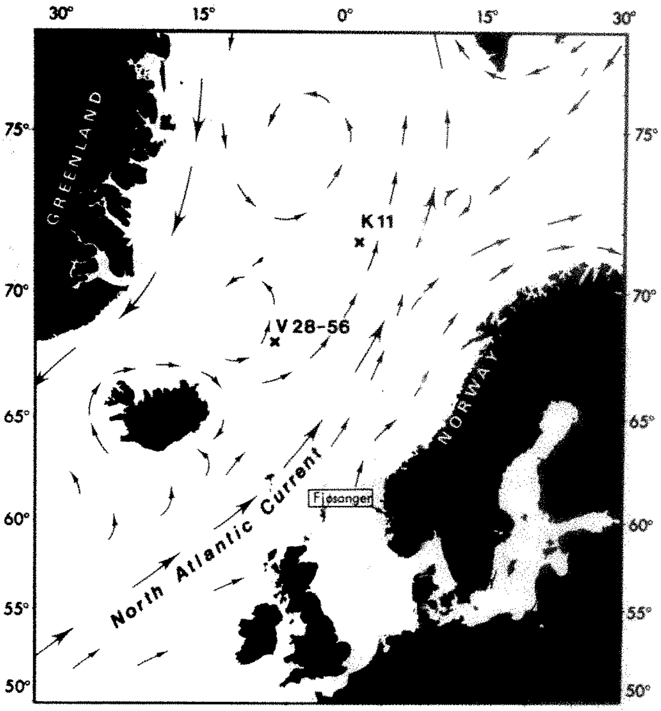

Fig. 1 Map of the Norwegian Sea and surrounding areas. The location of Fjøsanger and the cores mentioned in the text are indicated. The oceanic surface currents are also shown.

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Until recently it had been agreed that the Weichselian Stage immediately followed the Eemian. As the type area for the Weichsel glaciation is in Poland, some correlations are, however, necessary for this interpretation. It is also clear that glaciers reached Poland only during parts of the corresponding chronostratigraphical unit, the Weichselian Stage. If the Weichselian follows the Eemian, the type locality for the Eemian would also be the best boundary stratotype for the Eemian–Weichselian Stage boundary, and it has been used as such for a considerable time. We accept this definition based on the abundant evidence from North-west Europe for this sequence. Woillard[14] has suggested, however, that interglacials exist between the Eemian and the Weichselian. She carried out thorough studies of the most complete pollen sequence through the Upper Pleistocene in western Europe, the Grand Pile, France, and recognised that the Eemian was succeeded by two warm periods which she defined as interglacials St Germain I and II. The lower (St Germain I) is further divided into two warm phases, with a short cooling between.

St Germain I and II can be correlated with the Early Weichselian interstadials, the Amersfoort, Brørup[11,15] and Odderade[16]. The arguments for this correlation are: (1) their stratigraphical positions immediately above the Eemian are identical. (2) The vegetation during the St Germain I and II indicates a colder climate than does the Eemian vegetation at the same site. (3) Considering the southern location (47° 44′ N) of the Grand Pile, it is possible to correlate the vegetation during the St Germain I and II with the vegetation in North-west Europe during Amersfoort, Brørup and Odderade.

To avoid any confusion which may arise if the above statements about the Eemian and Weichselian should be shown to be erroneous, we have defined local chronostratigraphical units, which we subsequently correlate with the Eemian and the Weichselian (Fig. 2).

The Fjøsanger Interglacial, and its correlation with the Eemian

The Fjøsanger locality is situated along the shore of a small fiord, just outside the city of Bergen, western Norway (Fig. 1). Preliminary descriptions of the section have already been given[17,18], and a detailed description will be published else-

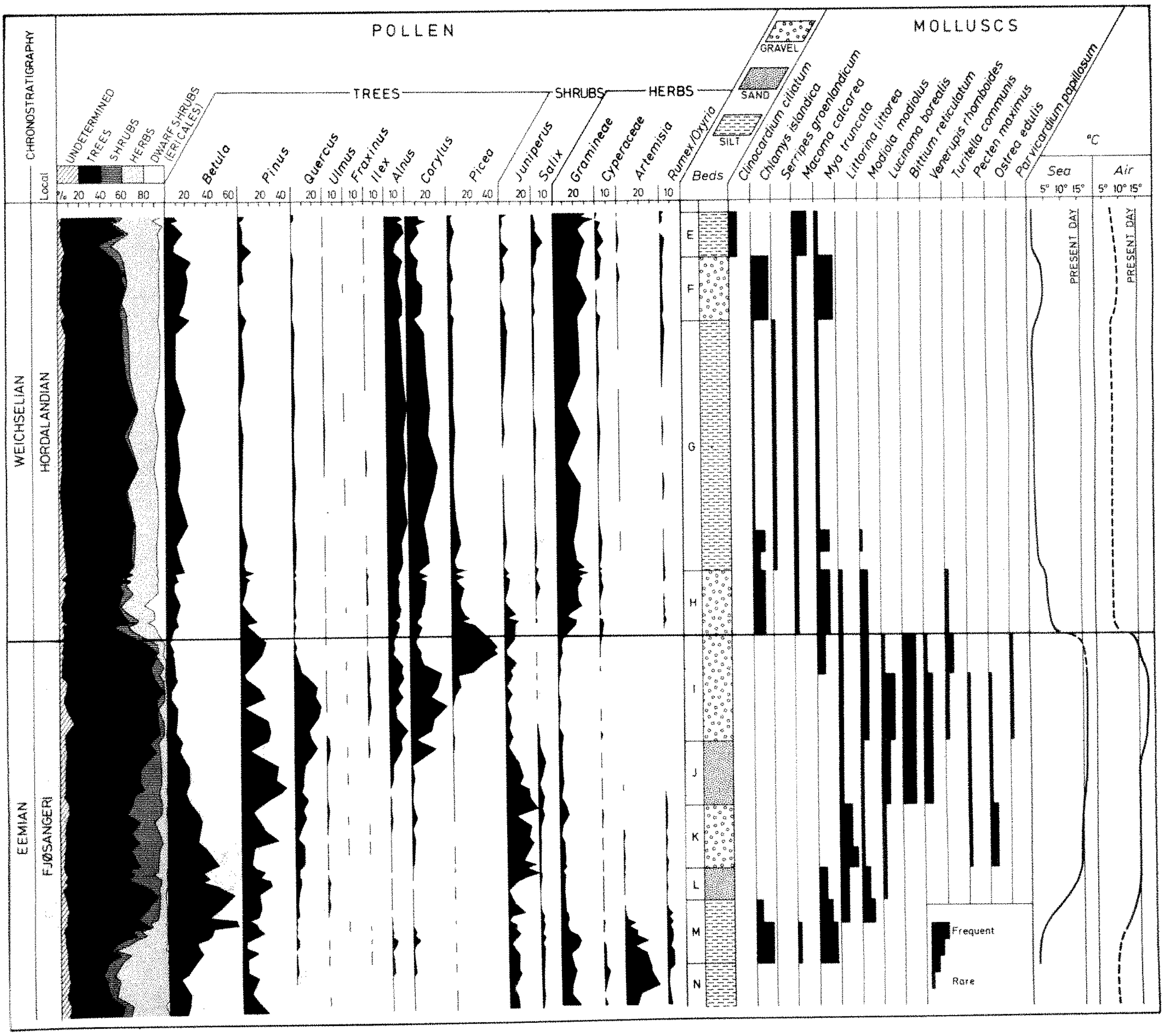

Fig. 2 The stratigraphy of the Fjøsanger site. The lithology of the beds is simplified and only the most important species of pollen and molluscs are shown. The temperature curves indicate mean air and sea temperatures for the warmest months, July and August respectively. The atmosphere curve is deduced from pollen data, the sea curve from molluscs and benthic foraminifera.

where. Two till horizons are located at the base of the sequence. Above these follows a sequence of marine sediments (beds N–E, Fig. 2), deposited in a fiord at depths of between 10 and 50 m during an interglacial and the earliest part of the following glacial period. The sediments consist of silt, sand and gravel (Fig. 2). However, even the coarse-grained sediments have a matrix of clay and silt, and both the benthic foraminifera and the pollen stratigraphy support an interpretation of continuous deposition of the clay–silt fraction throughout the marine sequence. The section is capped by a till.

We (in preparation) have defined the Fjøsangerian Stage with a lower boundary at the base of bed N (Fig. 2) and an upper boundary between beds I and H. The latter is also the lower boundary for the Hordalandian Stage.

The main criterion for the correlation of the Fjøsangerian Stage with the Eemian is the pollen stratigraphy (Fig. 2), the most significant features of which are: (1) the very distinct successional phases. (2) Early immigration of *Quercus*. (3) Development of a dense *Quercus–Corylus* forest, with very sparse occurrence of other QM-constituents. (4) *Alnus* increased at the same level as *Corylus*. (5) Late immigration of *Picea*, with a peak very late in the interglacial. The only main feature of the Eemian which is lacking is the *Carpinus* phase, which is almost certainly due to the northerly latitude of the Fjøsanger locality. The vegetation during the deposition of gravel I, indicates a mean summer temperature slightly higher than at present (Fig. 2).

At the type locality, the lower boundary for the Eemian Stage is placed at a horizon when a closed forest was established[12]. By comparison with the Holocene vegetational development it may be assumed that the spread of *Betula* woodland at Fjøsanger took place slightly later than at the type locality in the Netherlands. Actually, Fjøsanger was still covered by ice at the Weichselian–Holocene boundary. Bearing this in mind, we have placed the lower boundary for the Eemian immediately after the deglaciation of the site, and we assume that the error in this time correlation should not exceed some few hundred years.

We have correlated the Eemian–Weichselian boundary with the Fjøsangerian–Hordalandian boundary where the NAP-pollen increases from ~5% to 30%. This clearly represents a complete deforestation of the area, and we assume that all tree pollen types above this level, except possibly some *Betula*, are redeposited.

The molluscs and benthic foraminifera lead to very similar conclusions, and therefore only the mollusc diagram is presented here (Fig. 2). The fauna indicates cold polar water in the lower part of the Eemian (silt M, Fig. 2) and thereafter a rapid increase in temperature due to the appearance of the Atlantic Current. In beds I and J the fauna indicates warmer water than along the coast today, and also species not known from Norwegian waters were (for example, *Parvicardium papillosum*). As some of the mulluscspecimens are only referred to bed I, and the benthic foraminifera indicate a shallowing during the deposition of the upper part of this bed, it is difficult to detect a possible fall in temperature during the deposition of the upper part of bed I, but this must in any case have been very modest. From the deposition of bed I to that of bed H a drastic fall in temperature occurred, but the influence of the Atlantic Current is still detectable. In silt G, a polar fauna dominated, and during the deposition of this bed, glaciers were calving in the fiord. The fauna in gravel F indicates that the glaciers had retreated and this bed is assumed to correspond to an interstadial. Silt E represents another glaciomarine facies, above which the till follows. Whether or not a hiatus exists below the till is impossible to determine.

Three features of the Fjøsanger section are of major importance for correlation with the Norwegian Sea stratigraphy: (1) an oceanic circulation pattern similar to that of the present was established in the lower part of the Eemian. (2) During most of the Eemian, coastal waters were as warm, or warmer, than at present. (3) A drastic cooling of the sea water occurred at the Eemian–Weichselian boundary.

Correlation with the deep-sea record

Kellogg[3,19] studied cores from the Norwegian Sea. Mainly by means of two faunal indices (Fig. 3), he has shown that only once during the past 450,000 yr has the surface water of the Norwegian Sea been as warm, or warmer, than during the Holocene.

The $^{18}O/^{16}O$ ratio has been measured in two of the cores from the Norwegian Sea, and one core from the Denmark Straight[4,5]. The younger oxygen-isotope stages used in the world ocean stratigraphy were clearly recognised in these cores (Fig. 3).

The dominant factor determining the surface temperature of the sea both in the eastern Norwegian Sea and along the coast of Norway is the Atlantic Current. From a theoretical point of view, it is clear that faunas from the Norwegian coast, and the surface of the eastern Norwegian Sea, can be correlated by means of their temperature requirements. This is in accordance with knowledge of the present-day zoogeography, and also with geological correlations of the major change in the current pattern 10,000–13,000 yr ago[20–23].

The 'warm pulse' described by Kellogg can therefore be unambiguously correlated with the warm-water phase at Fjøsanger. The warm pulse in the Norwegian Sea appears at the same level as the oxygen-isotope stage 5e. As the warm-water phase at Fjøsanger occurred within the Eemian, the Eemian Stage can be correlated with the oxygen-isotope stage 5e.

Concerning a more precise correlation of the boundaries, the 6–5e boundary in the cores V 28–56 and K-11 (Fig. 3) is

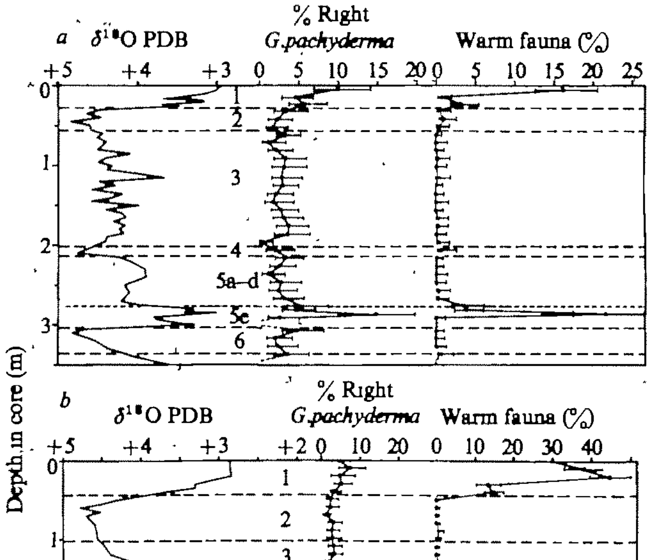

Fig. 3 The stratigraphy of two Norwegian-Sea cores (Fig. 1): *a*, K-11; *b*, V 28–56; according to Kellogg, Duplessy and Shackleton[4]. The curves represent (from the left): the $^{18}O/^{16}O$ curve; the oxygen-isotope stages (numbers), % right-coiling species (warm) of *Globigerina pachyderma*; and % warm-water foraminifera species.

significantly below the warm pulse. At Fjøsanger there is also a cold fauna in the lower Eemian. We have deduced, however, a high sedimentation rate for this part of the stratigraphy, and thus a short time period. This would indicate that the lower boundary for the Eemian is slightly above the 6–5e boundary.

In the cores from the Norwegian Sea (Fig. 3), the upper boundary of the warm pulse is very close to the 5e–5d boundary. At Fjøsanger the dramatic cooling of the seawater also occurred at the Eemian–Weichselian boundary, indicating that the Eemian–Weichselian boundary is nearly synchronous with the 5e–5d boundary.

These correlations are also supported by sea-level data from the North Sea area, for which Zagwijn[24] has given the best data. A major part of the transgression took place within the Eemian, indicating that the Eemian started long before the peak of the ^{18}O/^{16}O curve. Around the Eemian–Weichselian boundary, Zagwijn[24] found a drop in sea level of 32 m, of which half took place within the Eemian. Compared with the ^{18}O/^{16}O sea-level curve derived by Shackleton and Opdyke[1], this suggests that the Eemian–Weichselian and the 5e–5d boundaries are synchronous.

The sea-level data are also a very stong argument, independent of the main theme of this paper, that the Eemian should be correlated with 5e, and not a larger, or other part of stage 5.

The onset of the last glaciation

The forests at Fjøsanger during the later part of the Eemian indicate a climate which excluded the growth of any large ice-masses in Scandinavia. The drop in sea level during the Eemian thus indicates that the growth of ice sheets started earlier in North America and/or Antarctica than in Scandinavia.

At Fjøsanger the glaciomarine silt G (Fig. 2) confirms that glaciers approached Bergen early in the Weichselian. From the present day topography and climate, one can conclude that these first glaciers descended from mountains in the vicinity, and were not outlets from an inland-ice. These glaciers retreated at least once (bed F, Fig. 2) before Fjøsanger was covered by ice.

The vegetational succession during the upper part of the Eemian indicates, in our opinion, a climatic deterioration. However, the drastic fall in temperature at Fjøsanger occurred simultaneously in the sea and on land at the Eemian–Weichselian boundary. There can be no doubt that the cause of the sudden cooling was a weakening of the flow of the Atlantic Current into the Norwegian Sea. If the Atlantic Current was 'switched off' the sequence described would result, whether or not this event was preceded by, or caused by, a climatic deterioration.

The radiometric age of the Eemian

From the ^{14}C isotope enrichment method, the age of the early Weichselian interstadials has been estimated as 55,000–70,000 yr BP[25], and the Eemian is commonly assumed to be slightly older.

Another approach is correlation with tropical coral reef terraces, dated with the U-series methods. Shackleton and Matthews[26] showed that the oxygen-isotope stage 5e can be correlated with the Barbados III terrace, dated to 125,000 yr BP. Accepting the correlation of 5e with the Eemian, this age should also apply to the Eemian.

There is a significant discrepancy in the results from the two methods. At present we find the U-series ages most probable.

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Nucleotide sequence of a region of human mitochondrial DNA containing the precisely identified origin of replication

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A fragment of HeLa cell mitochondrial DNA containing the origin of replication has been sequenced. The precise position of the origin in this sequence has been identified by determining the nucleotide order in the 5′-end proximal portion of the heavy strand initiation fragment (7S DNA), and by aligning the two sequences.

MITOCHONDRIAL DNA synthesis in animal cells has been shown to have very distinctive features. In mouse L cells, where this process has been studied in most detail, DNA replication starts at a site located near the 5′-end of the 12S rRNA coding sequences with the displacement synthesis of a daughter H-strand, which grows in the direction away from the rRNA genes. When the nascent H-strand is at least two-thirds complete, synthesis of the complementary L-strand DNA begins at a point

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approximately 67% of the genome length from the H-strand origin, proceeding in the opposite direction, and ultimately replication is completed[1,2]. The same type of asymmetrical replication of mit-DNA has been observed in other animal cell systems such as HeLa cells, chick embryo, chick liver, rat liver, *Xenopus* cells and sea urchin oocytes[3]. However, the degree of asynchrony in the replication of the two strands varies considerably between different systems and also in the same system. Furthermore, in most systems investigated, including rat liver and rat hepatoma cells[4], sea urchin oocytes[5], *Drosophila melanogaster*[6] and HeLa cells (D. Carré and G. A., unpublished observations), a varying, though generally small, proportion of mit-DNA molecules has been observed to undergo a synchronous unidirectional replication of the two strands, resulting in totally duplex replication forms. The significance and possible regulation of this variability in the degree of asynchrony between displacement synthesis and complement synthesis in mit-DNA replication are unknown.

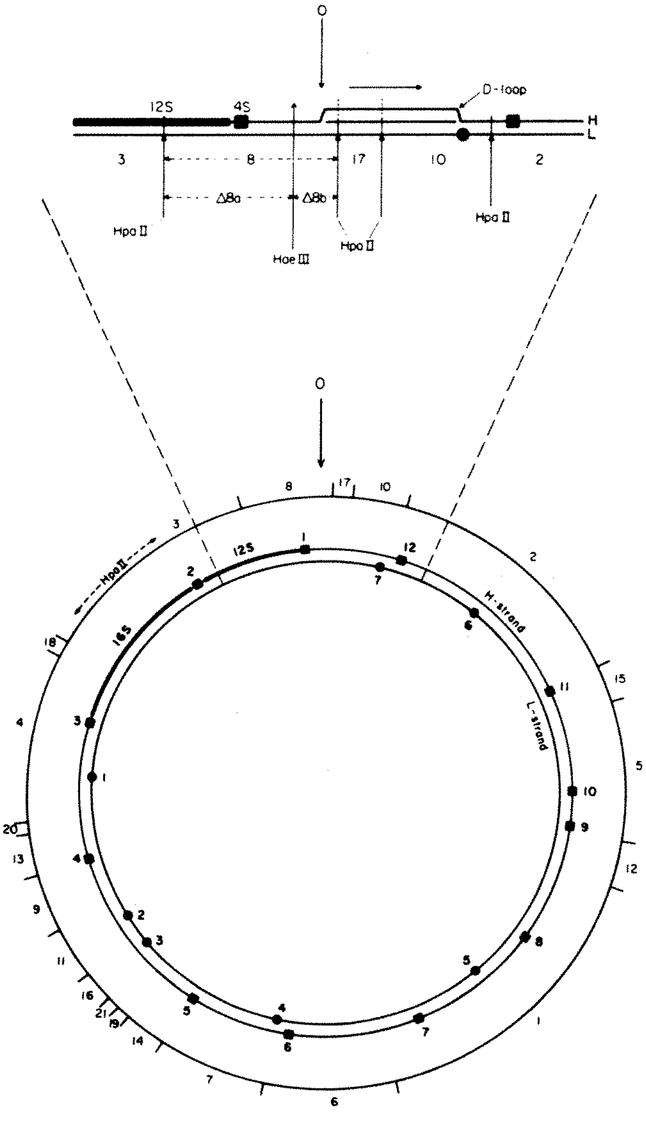

Fig. 1 Physical and genetic maps of HeLa cell mit-DNA. The restriction endonuclease map determined with the enzyme *Hpa*II[12] has been aligned with the *Eco*RI and *Hin*dIII maps[14] and with the map of the positions of the complementary sequences for the 12S and 16S rRNA on the H-strand and for 4S RNAs on the H(■) and L strands (●)[15]. In the upper part, a region of the *Hpa*II physical map of HeLa cell mit-DNA has been expanded to show the precise positions of the origin of replication (vertical arrow [marked O]) and of the D-loop. The rightward arrow indicates the direction of H-strand synthesis. H: heavy strand; L: light strand. The arrows marked *Hpa*II indicate *Hpa*II cleavage sites; the arrow marked *Hae*III indicates an *Hae*III site in *Hpa*II fragment 8 (modified from D.O. and G.A.[13]).

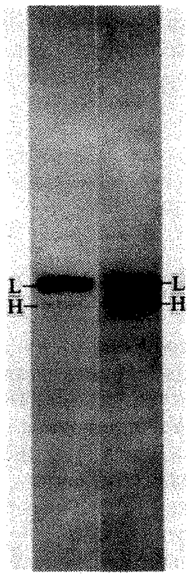

Fig. 2 Autoradiograph of the separated strands of 5′-end labelled fragment $\Delta 8b^{Hae}$. The restriction fragment $\Delta 8b^{Hae}$ was obtained by cleaving purified *Hpa*II fragment 8 with *Hae*III and electrophoresis of the digestion products on a 1.5% agarose gel in 0.032 M Tris, 0.02 M sodium acetate, 0.002 M EDTA, *p*H 7.4, for 4 h at 4 V cm^{-1}. The fragment was eluted by diffusion at 37 °C in 0.001 M Tris-HCl. *p*H 7.4, 0.001 M EDTA. In the left track, fragment $\Delta 8b^{Hae}$ was labelled, without prior dephosphorylation, with [γ-^{32}P]ATP and T4 polynucleotide kinase (PL Biochemicals) using the exchange reaction. In the right track, $\Delta 8b^{Hae}$ was dephosphorylated with 4 μg of bacterial alkaline phosphatase (Worthington BAPF) in 100 μl of 0.01 M Tris-HCl, *p*H 8.0, at 37 °C for 1 h (freed of divalent cations by treatment with BioRad Chelex 100)[19]. The phosphatase was removed by extraction with phenol–chloroform–isoamyl alcohol (50:50:1), or inactivated by the addition of nitrilotriacetic acid to 0.005 M at 25 °C[20]. The dephosphorylated 5′-ends were 'kinased' by the procedure described by Maxam and Gilbert[16], which included a heat denaturation step in the presence of 0.001 M spermidine. The labelled fragments were denatured in 0.001 M Tris-HCl, *p*H 7.4, 0.001 M EDTA for 5 min at 90 °C, quickly cooled in an ice bath and, after addition of a Ficoll–bromphenol blue mixture, electrophoresed on a 6% polyacrylamide (1 : 30 bisacrylamide) gel in 0.05 M Tris-borate, *p*H 8.3, 0.001 M EDTA. The gel was run at 4 °C for 15 h at 6 Vcm^{-1}. The two separated strands were excised from the gel and eluted by diffusion at 37 °C in 0.5 M ammonium acetate, 0.1% SDS, 10^{-4} M EDTA. In later experiments, *Hpa*II fragment 8 was cleaved with *Hae*III, and the products were directly kinased and subjected to strand separation as above.

Equally unique to mit-DNA of most animal cells investigated so far is the presence, in a substantial portion of the molecules, of a displacement loop (D-loop) near the origin of replication. This loop results from the synthesis of a short segment of H-strand DNA which displaces the parental H-strand[7]. These single-strand segments of DNA (referred to as 7S DNA because of their sedimentation coefficient in sucrose gradients) have recently been shown to exist as discrete size classes ranging between 500 and 700 nucleotides depending on the cell type. In mouse L cells, most of the 7S DNA molecules have a common 5′-end with a variability in length at the 3′-end[8], whereas, in human cells, the variability has been reported to be at the 5′-end[9] or at both ends[8]. In L cells there also seems to be a microheterogeneity at the 5′-end of each predominant size class[8]. Recent studies have shown that the 7S DNA is unstable, with a half life of about 1 h in L cells[10] and 2.8 h in human KB cells (D. Bogenhagen and D. Clayton, personal communication). Therefore, if 7S DNA is a primer for DNA replication, only a small fraction of the 7S DNA molecules can serve this role, as mit-DNA is known to be stable. The true function(s) of 7S DNA is still unknown. It may reflect a need for the cell to be able to recognise the origin or to continuously expose the single-stranded parental H-strand for initiation of transcription. Conceivably also, the D-loop may be involved in the recently discovered interaction between the mitochondrial inner membrane and the origin of mitochondrial DNA replication[11].

This article describes a sequence analysis of the region surrounding the origin of mit-DNA replication in HeLA cells. In these cells, by electron microscopic mapping of the D-loop with respect to certain restriction enzyme cutting sites, the origin of mit-DNA replication had been previously located in one of the three *Hpa*II fragments, 8, 10 or 17 (ref. 12). More recently, by using 7S DNA as a template for fragment-primed DNA synthesis by *Escherichia coli* DNA polymerase I and by carrying out a polyacrylamide gel electrophoretic analysis of the *in vitro* products after restriction enzyme cleavage, it has been possible to localise the position of the 5′-end of the 7S DNA in *Hpa*II fragment 8, at about 80 nucleotides from the *Hpa*II cutting site between fragments 8 and 17 (ref. 13). It has been further shown that *Hpa*II fragment 8 can be subcleaved by *Hae*III into two smaller fragments designated as $\Delta 8a^{Hae}$ (~560 base pairs) and

$\Delta 8b^{Hae}$ (~200 base pairs), and that the origin is contained in $\Delta 8b^{Hae}$ (ref. 13). This information, summarised in Fig. 1, has thus provided the foundation for the sequencing work described here.

Sequence analysis of the region of mit-DNA containing the origin of replication

The approach followed here to sequence the region of mit-DNA around the origin of replication involved isolating the restriction fragment $\Delta 8b^{Hae}$, labelling the 5′-ends with [γ-^{32}P]ATP and T4 polynucleotide kinase, separating the strands and sequencing both strands by the technique of Maxam and Gilbert[16]. As the fragment $\Delta 8b^{Hae}$ was known to be around 200 nucleotides long, we hoped that by ordering more than 100 nucleotides from each end, we could sequence the entire fragment with a substantial sequence overlap. Fragment $\Delta 8b^{Hae}$ was isolated by gel electrophoresis, eluted and subjected to two kinds of kinase reactions: (1) the exchange reaction[17,18], which does not require prior dephosphorylation of the 5′-terminus of the DNA, and (2) removal of the 5′-phosphate with bacterial alkaline phosphatase and then treatment with polynucleotide kinase[16]. Figure 2 shows the results of the kinase reactions on strand separation on a polyacrylamide gel. Significantly, in the DNA labelled by the exchange reaction, the faster migrating band contained only 15% as much radioactivity as the slower moving band, whereas DNA labelled after dephosphorylation revealed a faster migrating band labelled to the extent of about 65% of the slower band. As the incorporation into the slower migrating band was similar in the material labelled by the two procedures, in the present work, the DNA was routinely dephosphorylated before treatment with polynucleotide kinase.

After elution of the material in the two labelled DNA bands, aliquots of each sample were hybridised to an excess of either the heavy or the light strand of HeLa cell mit-DNA, incubated with S_1 nuclease, and the acid precipitability measured. The DNA from the faster migrating band hybridised to the extent of about 56% of the input c.p.m. to the H-strand and of 2% to the L-strand, whereas about 42% of the input c.p.m. in the material from the slower migrating band hybridised to the L-strand and 0.6% to the H-strand. The lack of complete hybridisation to mit-DNA of the two separated Δ8b strands is presumably due to the occurrence of some breaking at the ends of the hybrids, with the S_1 nuclease cleaving off a portion (40–50%) of the 5′-labelled nucleotides. On the basis of these data, we have designated the faster migrating DNA strand, $\Delta 8b^{Hae}(H)$, and the slower migrating DNA strand, $\Delta 8b^{Hae}$(L). It is not clear why the dephosphorylated and kinased $\Delta 8b^{Hae}$(H) contained a lower amount of radioactivity than $\Delta 8b^{Hae}$(L) (varying, in different experiments between 75% and 31%). This difference is probably due to incomplete dephosphorylation, or to renaturation and subsequent lower incorporation during kinase treatment at the flush end created by the *Hae*III enzyme relative to the staggered 5′-end produced by *Hpa*II, or to some other feature, such as secondary structure.

Sequence analysis of the labelled DNA strands was carried out by the Maxam and Gilbert procedure[16], using the G > A, A > C, C+T and C reactions. The sequence of nucleotides 2 to 25 was determined by fractionating the cleavage products on a 25% polyacrylamide–7 M urea gel; nucleotides 26 to 148 for $\Delta 8b^{Hae}$(L) and nucleotides 25 to 123 for $\Delta 8b^{Hae}$(H) were identified by fractionating the reaction products on 10% polyacrylamide–7 M urea gels (Fig. 3). The total length of the fragment was found to be 221 nucleotides, and thus it was possible to obtain an approximately 50 nucleotide overlap.

To complete the sequence, the end-labelled separated strands were digested exhaustively with endonuclease P_1, which generates 5′-mononucleotides[21], and the products were separated by one-dimensional chromatography on thin layer polyethylenimine strips. The results shown in Table 1 indicate that the 5′-terminal nucleotide of both the H and L strands is dCMP, as expected for the *Hpa*II and *Hae*III cutting sites. The complete sequence of the fragment $\Delta 8b^{Hae}$ is shown in Fig. 4.

Table 1 5′-terminal nucleotide analysis of $\Delta 8b^{Hae}$(*L*), $\Delta 8b^{Hae}$(H) and 7S DNA

	% of total ^{32}P c.p.m.*							
	dAMP	dCMP	dGMP	dTMP	AMP	CMP	GMP	UMP
$\Delta 8b^{Hae}$(L)	0.15	98.6	0.61	0.68				
$\Delta 8b^{Hae}$(H)	0.43	91.8	5.2	2.6				
7S DNA								
After isolation ·								
Expt 1	0.6	1.0	3.3	94.2	0.1	ND	ND	ND
Expt 2	1.0	1.4	4.4	93.1	ND	ND	ND	ND
In situ	2	5	3	89	ND	1	1	ND

5′-end-labelled $\Delta 8b^{Hae}$(L) and $\Delta 8b^{Hae}$(H) were dissolved in 15 μl 50 mM ammonium acetate, pH 5.4, and digested with 1 μg P_1 endonuclease from *Penicillium citrinum* for 1 h at 37 °C[21]. After addition of 10 nmol each of 5′-dAMP, dCMP, dGMP and dTMP, the mixture was applied to a PEI cellulose thin layer strip. After washing the strip in methanol, one-dimensional thin layer chromatography was carried out using 1 M acetic acid for 2 cm and then 1 M acetic acid–3 M LiCl(9 : 1) for 16 cm[22]. 7S DNA, with 15 μg of yeast tRNA carrier, was dissolved in 15 μl 50 mM ammonium acetate, pH 5.4. The mixture was digested with 2.5 μg of P_1 at 37 °C for 3 h. After addition of marker 5′-dNMPs, the digest was spotted onto a PEI cellulose plate and run in the first dimension as above. The plate was washed successively with Tris–methanol and methanol, and then run in a second dimension for 16 cm using 6 g $Na_2B_4O_7 \cdot 10H_2O$, 3 g H_3BO_3 and 25 ml ethylene glycol in 70 ml water[22]. All thin layer plates were examined by autoradiography sensitive enough to detect 1% of the input radioactivity (3 d.p.m. in a 1-mm^2 circle), and then the marker spots were visualised with a short-wave UV lamp and the spots cut out. They were eluted quantitatively with 1 ml of 0.02 M Tris-HCl, pH 7.4, 0.7 M $MgCl_2$ and counted in 10 ml of a Triton–xylene based scintillation fluid. ND, not detectable.

* The values refer to the radioactivity which migrated from the origin. The % of the input radioactivity recovered from the origin was, respectively, 0.5 and 4.9% for $\Delta 8b^{Hae}$(L) and $\Delta 8b^{Hae}$(H), not detectable and 1.2% for isolated 7S DNA (experiments 1 and 2, respectively) and 2.0% for the *in situ* labelled 7S DNA

Isolation and characterisation of 7S DNA

To precisely localise the origin of mitochondrial DNA replication in the $\Delta 8b^{Hae}$ fragment, the approach followed was to sequence the 5′-end portion of the 7S DNA and to align this sequence with that of $\Delta 8b^{Hae}$. Previous reports of 7S DNA size heterogeneity in human, mouse and other animal cells and also of an apparent 7S DNA 5′-end microheterogeneity in mouse L cells required that HeLa cell 7S DNA be first characterised in its size and 5′-termini.

Isolation of 7S DNA was carried out by the procedure of Kasamatsu *et al.*[7], in which the 7S DNA molecules are released from closed circular mit-DNA by brief heat treatment and then separated by sucrose gradient centrifugation. The material in the 7S DNA peak was further purified from RNA contaminants by RNase digestion or nitrocellulose chromatography[23], and then isolated by polyacrylamide or agarose gel electrophoresis in denaturing conditions.

Figure 5 illustrates the purification procedures used. Slot 2 shows the pattern obtained by electrophoresing through a 4% polyacrylamide–7M urea gel a sample of sucrose gradient-isolated 7S DNA labelled *in vitro* at the 5′-end with [γ-^{32}P]ATP and T4 polynucleotide kinase. A prominent band (indicated as 1) is seen migrating at a position corresponding to a length of about 680 nucleotides (estimated by comparison with marker HeLa cell *Hpa*II fragments). A fainter band (indicated as 2), corresponding to a size of about 630 nucleotides, can also be seen. The further purification achieved by nitrocellulose chromatography is shown in slot 3, where the discrete and heterogeneous components visible in the lower part of the gel and presumably arising from RNA contaminants have been

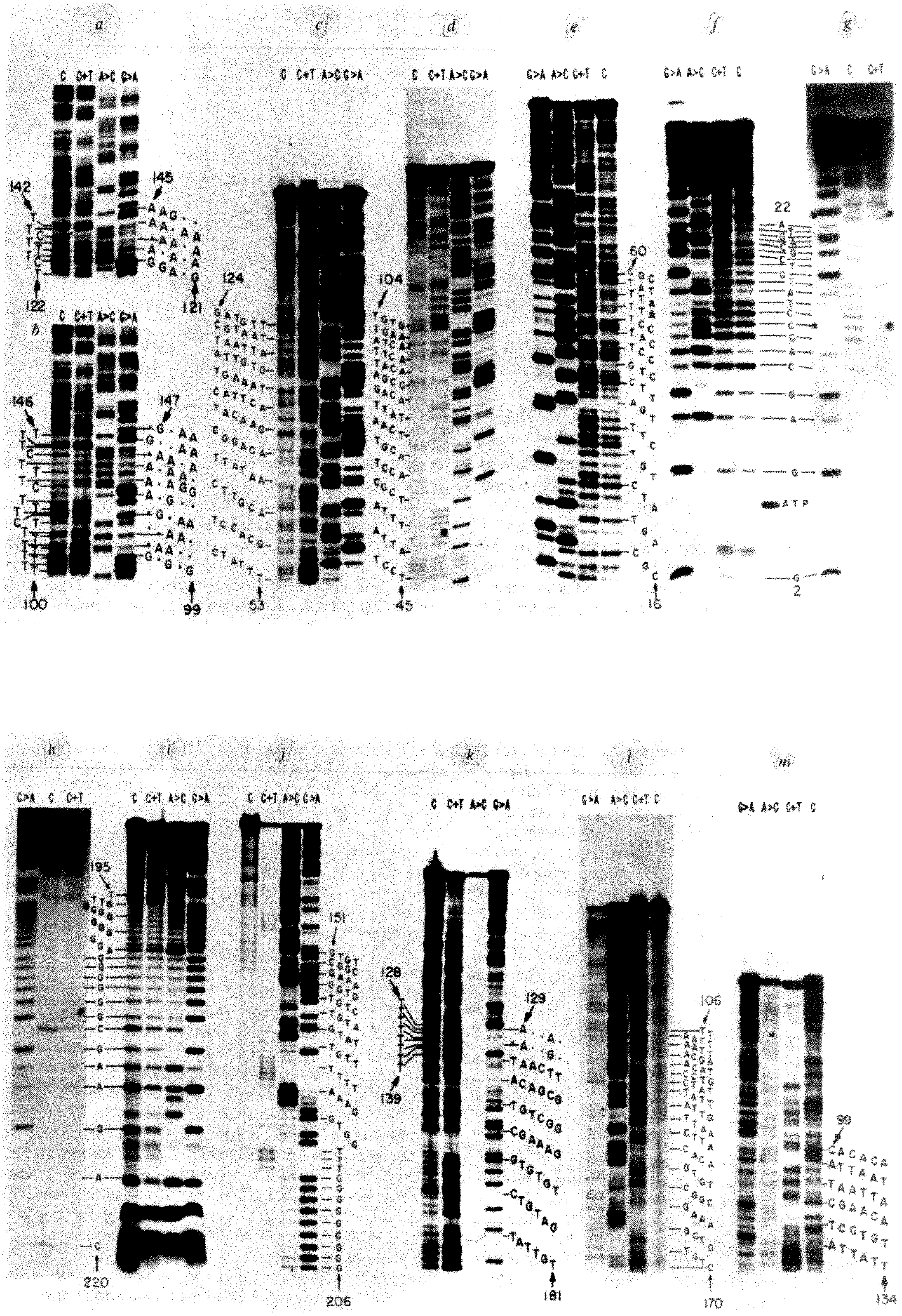

Fig. 3 Representative autoradiographs of sequencing gels of $\Delta 8b^{Hae}$(L) (*a–g*) and $\Delta 8b^{Hae}$(H) (*h–m*). The DNA strands, labelled at the 5′-end with [γ-^{32}P]ATP and polynucleotide kinase after alkaline phosphatase treatment (see text), were sequenced using the chemical methods of Maxam and Gilbert. The sequence reactions used and the times of reaction for the base modification step were G > A (10 min), A > C (10 min), G + T (15 min) and C (15 min). The degradation products were electrophoresed at 1,000 V for *a*, 15.5; *b*, 13; *m*, 12; *c*, *l*, 8.5; *d*, *k*, 7.25, *f–i*, 7 and *e*, *j*, 4 h on either 10% polyacrylamide (1 : 20 bisacrylamide)–7 M urea gels (*a–e*, *j–m*) or 25% polyacrylamide (1 : 30 bisacrylamide)–7 M urea gels (*f–i*) (40 × 30 × 0.15 cm) in 0.05 M Tris-borate, *p*H 8.3, 0.001 M EDTA, that had been pre-electrophoresed for 2 and 3 h, respectively, at 900 V. The gels were wrapped with Saran wrap and exposed at −70 °C using pre-fogged Kodak X-Omat XR-5 film with a DuPont Cronex Lightning-Plus intensifying screen.

```
        5'  C C A G A A G C G G G G G G A G G G G G G G G G T T T G G T G G A A A T T T T T T
        3'  G G T C T T C G C C C C C C T C C C C C C C C C A A A C C A C C T T T A A A A A A
             220                 210                 200                 190              181


G T T A T G A T G T C T G T G T G G A A A G C G G C T G T G C G A C A T T C A A T T G T T A T T A T T A T G T C C T A C
C A A T A C T A C A G A C A C A C C T T T C G C C G A C A C G C T G T A A G T T A A C A A T A A T A A T A C A G G A T G
180                 170                 160                 150                 140                 130               121
                                                              O
                                                              |
                                                              ↓
                                                          5'  T G T T C G C C T G T A A T A T T G A A C G T A C G --   7S DNA
A A G C A T T A A T T A A T T A A C A C A C T T T A G T A A G T A T G T T C G C C T G T A A T A T T G A A C G T A G G T
T T C G T A A T T A A T T A A T T G T G T G A A A T C A T T C A T A C A A G C G G A C A T T A T A A C T T G C A T C C A
120                 110                 100                 90                  80                  70                 61


G C G A T A A A T A A T A G G A T G A G G C A G G A A T C A A A G A C A G A T A C T G C G A C A T A G G G T G C T C     3'   H strand
C G C T A T T T A T T A T C C T A C T C C G T C C T T A G T T T C T G T C T A T G A C G C T G T A T C C C A C G A G G C   5'   L strand
60                  50                  40                  30                  20                  10                  1
```

Fig. 4 Nucleotide sequence of the fragment $\Delta 8b^{Hae}$ and of the 5'-terminal segment of 7S DNA.

eliminated. Slot 4 shows the electrophoretic pattern obtained with 7S DNA prepared, using the isolation procedure described above, from cells labelled *in vivo* with ^{32}P-orthophosphate. The pattern observed is very similar to that of the *in vitro* labelled material. Here, in addition to the two above mentioned bands, there is also a third faint band migrating at a position corresponding to a size of about 590 nucleotides. These observations on *in vivo* labelled material indicate that the appearance of the minor 7S DNA components is not due to artefacts introduced by the *in vitro* labelling conditions.

Comparison of the electrophoretic patterns of sucrose gradient-purified 7S DNA with those published by Brown *et al.*[9] and Gillum and Clayton[8] suggests that the major and the minor bands observed here correspond to the three major forms of 7S DNA detected by these authors in several human cell types. However, the relative intensities of the bands observed by the cited authors and those shown here are strikingly different. Instead of three components in approximately equal amounts, our material shows a preponderance of the largest, 680 nucleotide long, component. To exclude the possibility that this difference resulted from losses or degradation occurring during purification of the 7S DNA, the *in situ* labelling technique used by Gillum and Clayton[8] was followed. In this procedure, the 7S DNA is labelled without prior separation from closed circular mit-DNA. In Fig. 5, slot 5, where the results of this experiment are presented, the electrophoretic pattern seen is very similar to that of the *in vitro* labelled samples with the 680 nucleotide long component again being strongly predominant. Densitometric analysis of these autoradiograms indicate that this component represents 70–80% of the total 7S DNA in preparations labelled *in situ*, and more than 95% in preparations labelled after sucrose gradient isolation, assuming equal efficiency of *in vitro* labelling of the various components.

The fact that the relative intensities of the bands produced by the two labelling methods were comparable did not rule out the possibility that the difference in mobility between the 7S DNA components might be due to some factor other than heterogeneity in length. However, treatment with RNase (Fig. 5, slot 4) or pronase (in the presence of SDS) before electrophoresis failed to alter the observed number, relative abundance, or electrophoretic mobility of these components, thereby excluding any possible role of a covalently linked RNA primer or of DNA-associated protein in generating this phenomenon. In addition, when the *in situ* labelled component 2 and the *in vitro* labelled component 1 were isolated from the urea gel, heat denatured and re-run through a second urea gel in the same conditions (Fig. 5, slots 6 and 7, respectively), or through an agarose–CH_3HgOH gel[26] (not shown), they maintained the same relative electrophoretic mobility. The above results support the conclusion that the multiple bands arise from discrete length differences.

To investigate the possibility of 5'-end microheterogeneity (which would not have been detected by the above electrophoretic analysis), sucrose-gradient-isolated 7S DNA was labelled at the 5'-end with [γ-^{32}P]ATP and T4 polynucleotide kinase and subjected to digestion with the *Hpa*II restriction enzyme. (*Hpa*II is known to cleave single-stranded DNA[27], and previous experiments in this laboratory had indicated that the *Hpa*II site present in the single stranded portion of the D-loop (which is equivalent in sequences to the 7S DNA) is cut at a low frequency in standard digestion conditions[13].) The digest was analysed on a 7.5% polyacrylamide–7M urea gel; the results obtained (not shown) indicated that a small percentage of the 7S DNA molecules had been cut at a position corresponding to a distance of ~86 nucleotides from the labelled 5'-end (as estimated from the migration of the small fragment thus produced relative to that of denatured ΦX174 RF DNA *Hae*III fragments)[28]. As the resolving power of the gel electrophoresis used would have shown a difference of 1 or 2 bases in length, the above results indicate that the 5'-end of the main 7S DNA component occurs at a unique position within the mitochondrial genome. To obtain further evidence on this question, a 5'-end base analysis was carried out. 7S DNA was dephosphorylated at the 5'-end by alkaline phosphatase either *in situ* or *in vitro* after sucrose gradient isolation, labelled with [γ-^{32}P]ATP and polynucleotide kinase, purified by nitrocellulose chromatography and electrophoresed through a 4% acrylamide–7M urea gel. The main component was eluted from the gel and subjected to digestion with endonuclease P_1. The products were analysed by two-dimensional chromatography on polyethylenimine thin layer plates, and the results are given in Table 1. In the *in vitro* labelled sample, more than 90% of the counts recovered are represented by dTMP, confirming the conclusion that the 5'-end of the predominant HeLa cell 7S DNA component is unique. Furthermore, no radioactivity, or only traces of radioactivity, were detected in ribonucleotides. The results obtained for the *in situ* labelled sample are very similar, although a slightly larger proportion of radioactivity seems to be associated with deoxyribonucleotides other than dTMP; this may reflect the lower degree of purification of *in situ* labelled 7S DNA due to the omission of the sucrose gradient purification step.

Sequence analysis of 7S DNA

The main 7S DNA component was subjected to alkaline phosphatase treatment and the kinase reaction either *in situ* or after sucrose gradient purification, passed through a nitrocellulose column and then run on a polyacrylamide–urea gel. After elution of the 7S DNA from the gel, it was submitted to the Maxam–Gilbert sequencing reactions.

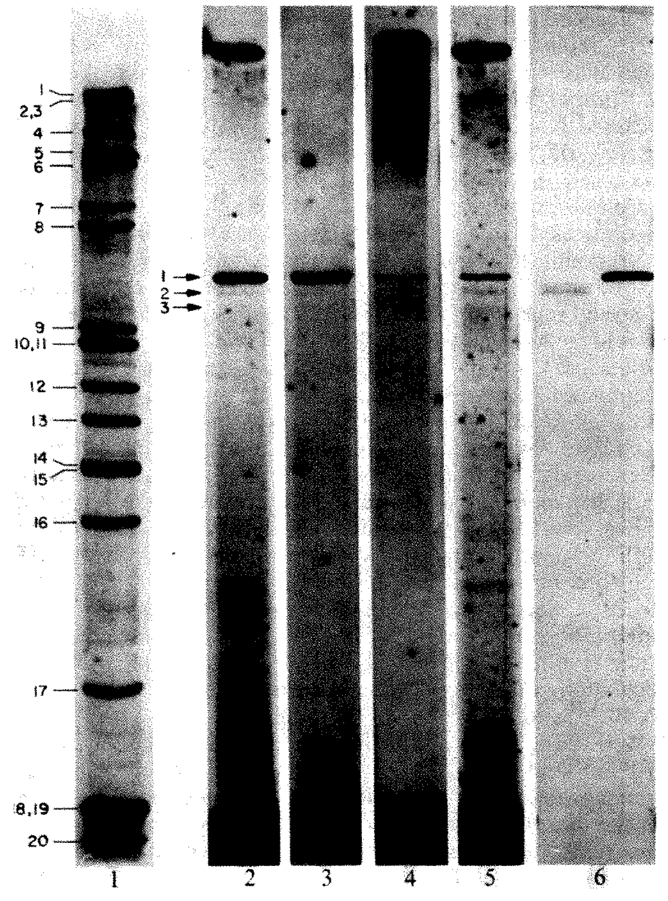

Fig. 5 Autoradiographs, after electrophoresis through 4% polyacrylamide–7 M urea gels, of HeLa cell 7S DNA labelled *in vitro* or *in vivo* and purified in different ways. All samples, in 0.001 M Tris-HCl, *p*H 7.4, 0.001 M EDTA, were adjusted to 6 M urea, heated at 100 °C for 1 min and loaded on a 4% polyacrylamide (1 : 30 bisacrylamide)–7 M urea slab gel. Electrophoresis was carried out at 5 V cm^{-1} for 15 h. *Slot 1*: *Hpa*II digest of HeLa cell mit-DNA labelled *in vitro* with [α-^{32}P]dNTP and *E. coli* DNA polymerase I[12]. *Slots 2 and 3*: 7S DNA was released from mit-DNA by brief heating, isolated by sucrose gradient centrifugation[7], and labelled *in vitro* at the 5′-end as follows: A sample of ~0.2 pmol of 7S DNA (estimated by assuming a proportion of about 10% D-loop containing molecules in HeLa cell mit-DNA preparations[24], and a complete recovery of the isolated 7S DNA) was dephosphorylated and 5′-end labelled as described in Fig. 2 legend. After ethanol precipitation, the pellet was dissolved in 0.001 M Tris-HCl, *p*H 7.4, 0.001 M EDTA. A portion of the sample was analysed directly (slot 2) and a portion was further purified by nitrocellulose (NC) chromatography. For this the sample (50 μl) was adjusted to 0.5 M KCl and passed through a 1.5 × 0.3 cm nitrocellulose column (Hercules, prepared as described by Boezi and Armstrong[25]) equilibrated with 0.5 M KCl, 0.01 M Tris-HCl, *p*H 7.4. The column was washed with equilibration buffer to remove all the unbound radioactivity, then eluted with 0.001 M Tris-HCl, *p*H 7.4, 0.001 M EDTA (slot 3). *Slot 4*: 7S DNA from 1.5×10^{8} cells labelled *in vivo* with ^{32}P-orthophosphate[12] was isolated by sucrose gradient centrifugation and run on gel after treatment with pancreatic RNase (40 μg ml^{-1}, 10 min at 25 °C). *Slot 5*: 7S DNA labelled *in situ* following the procedure of Gillum and Clayton[8]: a sample of 10 μg mit-DNA in 100 μl 0.01 M Tris-HCl, *p*H 8.5, 0.01 M NaCl, was incubated with 0.05 μg calf intestine alkaline phosphatase (Boehringer Mannheim) for 1 h at 57 °C, and then labelled with [γ-^{32}P]ATP and T4 polynucleotide kinase. *Slots 6 and 7*: 7S DNA components 2 and 1, which had been labelled at the 5′-end *in situ* or after sucrose gradient isolation, respectively, then purified by nitrocellulose chromatography, and run through a 4% polyacrylamide–7 M urea gel, were eluted and rerun in the same conditions.

Figure 6 shows a sequencing gel of 5′-end labelled, sucrose gradient-purified 7S DNA. A sequence of around 25 nucleotides could be determined unambiguously. This sequence is identical to the portion of the sequence of $\Delta8\mathrm{b}^{Hae}$(H) from residue 87 to residue 63. This alignment allowed the positioning of the 5′-end of 7S DNA to correspond with residue 87 of $\Delta8\mathrm{b}^{Hae}$. Sequencing experiments using *in situ* labelled 7S DNA gave identical results[28].

Discussion

In the experiments reported here, we have precisely located the origin of HeLa cell mit-DNA synthesis in the *Hpa*II map of this DNA and examined the sequence surrounding it. The location of the 5′-end of the 7S DNA, determined by sequence analysis to be at 87 nucleotides from the *Hpa*II cutting site between fragments 8 and 17, is in excellent agreement with the 7S DNA priming experiments that indicated the origin to be at approximately 80 nucleotides from the *Hpa*II site, and with the experiments in which 5′-end labelled 7S DNA was cut with *Hpa*II giving a labelled DNA fragment about 86 nucleotides long. The size of $\Delta8\mathrm{b}^{Hae}$ is 221 nucleotide pairs, which is about 10% longer than the value that was earlier derived from its electrophoretic mobility in agarose gels relative to that of *Hpa*II fragments.

There are several interesting features of the $\Delta8\mathrm{b}^{Hae}$ sequence (Fig. 4), although their significance is unclear. Beginning at nucleotide 215 and proceeding clockwise (the clockwise direction is here defined as the direction of H-strand synthesis), there is a stretch of 17 G·C base pairs in a row with an A·T pair interruption at position 207. Furthermore, there is a strong purine bias on the H-strand. Nearby, from position 189 to 181, there is a stretch of 9 A·T base pairs in succession. There is another A·T base pair stretch running from position 137 to 128, and, contained within it, an 8 nucleotide true palindrome from position 137 to 130. Closer to the origin is a 13 A·T base pair stretch which contains two true palindromes (113–104 and 116–105) and a sequence with a twofold axis of symmetry (115–104). Repeated sequences are relatively rare; the sequence $\begin{matrix}5'\,\mathrm{TTGTTAT}\,3'\\3'\,\mathrm{AACAATA}\,5'\end{matrix}$ at nucleotides 140–134 is repeated at positions 182–176, and the sequence $\begin{matrix}5'\,\mathrm{GTGGAAA}\,3'\\3'\,\mathrm{CACCTTT}\,5'\end{matrix}$ is found at nucleotides 166–160 and at 193–187. The sequence

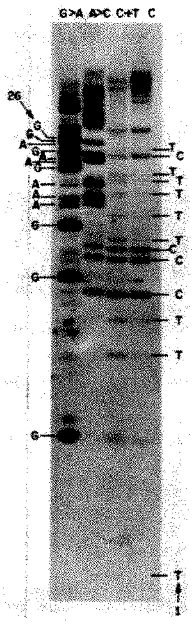

Fig. 6 Representative autoradiograph of 7S DNA sequencing gel. Sucrose gradient isolated 7S DNA was dephosphorylated and 5′-end labelled, as detailed in Fig. 2 legend, purified by nitrocellulose chromatography and then run through a 4% polyacrylamide–7 M urea gel. The main component (1) was eluted and subjected to the sequencing reactions G > A, A > C, C + T and C. Due to the low amount of radioactivity in the 7S DNA preparations, the base modification reactions were carried out for a time 10 times longer than used for the $\Delta8\mathrm{b}^{Hae}$ strands (legend to Fig. 3). This extended reaction time resulted in a decreased mobility of the products in the A > C reaction. The cleavage products were electrophoresed on a 25% polyacrylamide–7 M urea gel for 6.3 h.

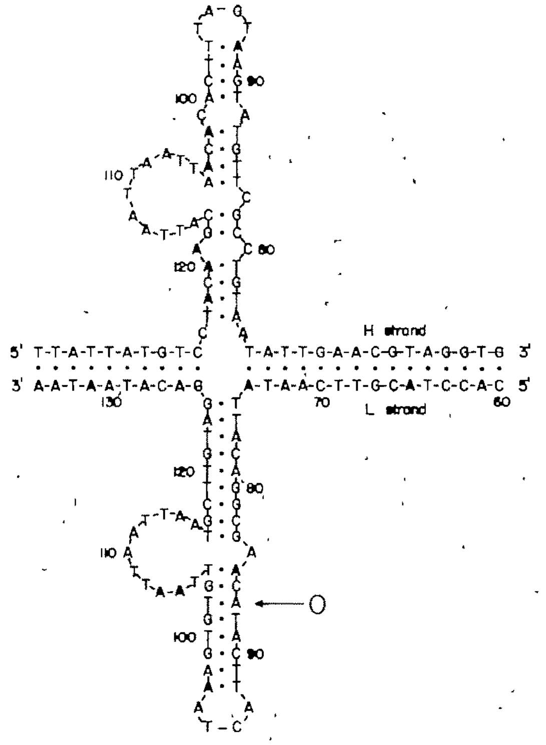

Fig. 7 Possible secondary structure of the region of fragment $\Delta 8b^{Hae}$ surrounding the origin of replication. The arrow (O) points to the nucleotide in the L-strand which is complementary to the 5′-end nucleotide of 7S DNA. The ΔG values of the L-strand and of the H-strand portions of the structure were calculated on the basis of the thermodynamic data for Watson–Crick nearest-neighbour sequences, loops, mismatches, and G·U pairs, as compiled for ribonucleic acids (see text) The free energies of base-pair addition were reduced by about 15% whenever the base-pair doublet included a G·C pair, in order to correct for the greater stability of the rG·rC pair as compared to dG·dC[34] In view of the many assumptions underlying these calculations, the ΔG values reported in the text have to be considered only as rough indicators of stability.

$$\begin{matrix}5'\,\mathrm{TATGATG}\,3'\\3'\,\mathrm{ATACTAC}\,5'\end{matrix}$$, often found to be associated with promoter sites[29], is located at nucleotides 178 to 172 and might possibly be associated with an L-strand promoter site.

The origin itself is preceded, going in a clockwise direction, by a 29-nucleotide long A·T rich region that extends 3 nucleotides past the origin, is followed by a 6-nucleotide G·C rich region and then by a 10-nucleotide long A·T rich region. A·T rich sequences have previously been noted near other origins of DNA replications; however, we do not detect any sequence characteristics in common with the other origins of replication and primer RNA–DNA junctions that have been accurately localised (ColE1[30] RNA–DNA junction, ΦX174 viral strand origin[31], fD[32] and G4 complementary strand[33] origins) nor with the L-cell 7S DNA sequence[8].

Computer analysis of the sequence data indicates a theoretically stable hairpin structure that could form when the DNA double helix is unwound, as occurs during DNA synthesis. As shown in Fig. 7, this looped structure contains the origin of H-strand replication and an interior A·T rich loop, 12 nucleotides long. As calculated using the rules of Tinoco *et al.*[35,36] and Gralla and Crothers[37], the ΔG of the L-strand hairpin structure is approximately $-13\ \mathrm{kcal\ mol^{-1}}$ and that of the H-strand hairpin is approximately $-5\ \mathrm{kcal\ mol^{-1}}$, with the difference being due to two A·C pair mismatches in the H-strand stem. Although this secondary structure is probably thermodynamically stable at 25 °C in a moderate ionic strength buffer at neutral *p*H, it is not known whether it is ever formed *in vivo*. However, the presence of the origin in the hairpin at only two nucleotides from the A·T rich interior loop is suggestive of a functional role for this structure. Secondary structure loops have been strongly implicated in the initiation of phage fD[32] and G4[33] complementary strand synthesis, and may be involved in the initiation and regulation of DNA replication in the mammalian papovaviruses SV40[38,39] and BKV[40].

We have not detected any ribonucleotides covalently attached to the 5′-end of the 7S DNA that might represent an RNA primer or residues of it. Clayton *et al.*[8] have presented evidence for the presence of an RNA primer in mouse L cell 7S DNA. The reason for this difference is unknown. In HeLa cell 7S DNA, we have also observed a greater homogeneity in size than previously reported in 7S DNA from other human cell types and other organisms. Nor have we detected the 5′-end microheterogeneity described in these systems. Our sequence analysis has not revealed any repetition of sequence or other structural features that might point to the existence of multiple initiation points, thereby explaining the existence of the multiple size classes. Instead, we think that the discrete size classes and the relative amounts of 7S DNA contained in each of them, the 5′-end microheterogeneity and the presence of RNA primer residues may reflect various rates or pathways of 7S DNA processing which could be dependent on cell type and/or physiological conditions.

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letters

Upper limits for γ-ray bursts from primordial black holes

PAGE AND HAWKING[1] have predicted that primordial black holes of mass $\sim 10^{15}$ g will evaporate, reaching an explosive stage at a mass which depends on the nuclear processes occurring. For the composite particle model 10^{34} erg of γ rays will be produced at energies of 100–1,000 Mev. For the elementary particle model 10^{30} γ rays of energy 5×10^{12} eV are expected. We have already set upper limits[2,3] for the rate of primordial black hole explosions in the Galaxy assuming the composite particle model. We report here upper limits from two other sets of data, assuming the elementary particle model and using the atmospheric Cerenkov technique as before.

In the first data set the Mt Hopkins 10-m reflector[4] was used with a field of view of 1°. Approximately 112 h of observation were analysed. As the detector has an energy threshold of 10^{11} eV it will respond to 5×10^{12} eV primaries over a large area. Events were recorded on analogue magnetic tapes together with a standard frequency and time markers. They were analysed by reconstituting the standard frequency with a phase-locked loop, and after scaling, deriving a 0.1 s clock signal. The number of events recorded in each successive 0.1 s interval was determined with a mini-computer and a distribution derived. The characteristic signature of a γ-ray burst would be a group of individual showers occurring together within 0.1 s.

At five points in the data bursts were observed which were incompatible with random Poisson expectation. Four of these were very large and had durations of 2–11 s. They were probably local in character and due to man-made interference. The fifth event was just above the burst threshold (12 showers) and lasted 0.1 s. Without a distant coincident detector this event could not be ascribed to a cosmic origin. We therefore derived an upper limit at the 99% confidence level of 6.1 events in 112 h, or 477 events yr^{-1}.

The collection area of showers of 5×10^{12} eV was estimated at 5×10^{9} cm^2 and the solid angle was taken conservatively at the geometric value of 2.4×10^{-4} sr. Twelve showers were required for a detectable burst, corresponding to 96 erg and giving a sensitivity of 1.9×10^{-8} erg cm^{-2}. The maximum detectable distance r is then given by $4\pi r^2 = 8\times10^{30}/1.9\times10^{-8}\ \text{cm}^2$, hence $r = 5.7\times10^{18}$ cm $= 1.9$ pc. The sensitive volume $V = \Omega r^3/3 = 5.5\times10^{-4}\ \text{pc}^3$. The upper limit for the explosion rate is then 8.7×10^{5} explosions $\text{pc}^{-3}\ \text{yr}^{-1}$.

In the second data set[5] two 1.5-m reflectors were used in coincidence aligned parallel to each other at varying zenith angles. The full field of view was 5°; 78 h of observation were obtained. The normal counting rate was about 300 showers min^{-1} at the zenith. The energy threshold for the system was 3×10^{12} eV and the collection area for 5×10^{12} eV γ-rays estimated at $3\times10^{9}\ \text{cm}^2$. Events were recorded on analogue magnetic tape as before.

No groups of events were recorded beyond Poisson expectation. Seven events would be required for a statistically detectable burst, corresponding at 5×10^{12} eV to 56 erg and giving a sensitivity of 1.8×10^{-8} erg cm^{-2}. The maximum detectable distance r is then given by $4\pi r^2 = 8\times10^{30}/1.8\times10^{-8}\ \text{cm}^2$ hence $r = 5.9\times10^{18}$ cm $= 2.0$ pc. The sensitive volume $V = \Omega r^3/3 = 1.6\times10^{-2}\ \text{pc}^3$. The upper limit at the 99% confidence level for zero bursts observed is 4.3 bursts in 78 h or 483 yr^{-1}. The upper limit for the explosion rate is then 3.0×10^{4} explosions $\text{pc}^{-3}\ \text{yr}^{-1}$.

Using particle detectors at sea level with a higher energy threshold, which required some assumptions about the primordial black hole emission spectrum, Fegan *et al.*[6] have found lower limits than those reported here. A further improvement in the Cerenkov method would require the use of one or more detectors with considerably larger collection area, and if possible, a wider field of view. A limit of about 1 explosion $\text{pc}^{-3}\ \text{yr}^{-1}$ is set irrespective of the nuclear model assumed, by observations of the isotropic γ-ray spectrum[1]. While the limit set with the Cerenkov technique assuming the composite particle model is only 4×10^{-2} explosions $\text{pc}^{-3}\ \text{yr}^{-1}$ (ref. 3), it seems likely that Cerenkov, particle, or satellite γ-ray detectors will probably not produce limits lower than about 10^{3} explosions $\text{pc}^{-3}\ \text{yr}^{-1}$ when the elementary particle model is assumed.

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A non-singular quantum Universe

CLASSICAL relativistic singularities remain one of the most puzzling problems of contemporary physics. Although no consensus of opinion has been attained on their status, a great deal of work has been devoted to the search for physical mechanisms capable of eliminating the cosmological singularities, whose existence follows from the theorems of Hawking and Penrose[1,2]. The alternative view that the initial singularity of big-bang models is essential for the coming into being of the Universe, has also been advocated[3,4]. Some suggestions of cosmological singularity avoidance have recently come to light, in the 'classical' quantum regime (from 10^{-43}–10^{-23} s) (refs 5–8) or even without resorting to quantum effects[9]. The quantum gravitational period ($t < 10^{-43}$ s) remains potentially very interesting for searching for singularity avoidance mechanisms. An interesting approach is that of quantum cosmology, based on the hamiltonian formulation of general relativity (Dirac's[10] or Arnowitt, Deser and Misner's[11] (ADM) method). This approach was initiated by De Witt[12] and then followed by Misner[13] and many others (see refs 14,15) and is used here to derive a non-singular quantum cosmological model.

Much of the work done in quantum cosmology has been concerned with empty models. De Witt[12], however, in his particles treatment of a closed Friedmann Robertson-Walker (FRW) Universe, introduced a cloud of dust particles to support this metric. The initial singularity is avoided artificially in this model by postulating the existence of an infinitely steep wall at $R = 0$, (R is the 'radius' of FRW Universe) to exclude negative values of R. This choice forces the quantum wave function of the Universe to be zero at $R = 0$.

Misner's[13] pioneering work about quantum vacuum homogeneous models (Bianchi I and IX Universes), based on the ADM formalism, shows that the quantum gravitational fluctuations offer these models no escape from the classical singularity. The same conclusion seems to be true for the Bianchi Universes filled with a perfect gas with an equation of state of the type $p = (\gamma - 1)\rho$ (p and ρ denote respectively the pressure and total density of matter and γ is a constant between 1 and 2) if the matter is described by Ryan's [14] lagrangian which introduces no additional dynamical degree of freedom for the perfect gas. (Units are chosen so that $c = 1$, $G = 1$ and $h/2\pi = 1$.)

On the other hand, Schutz[16,17] has built a hamiltonian theory of the relativistic perfect fluid, based on the velocity-potential version of perfect-fluid hydrodynamics of Seliger and Whitham[18]. Schutz's canonical formalism introduces as dynamical degrees of freedom for the fluid the velocity potentials.

Lund[19] has used Schutz's formalism to study the effects of quantum gravitational fluctuations on the final issue of collapse of balls of dust ($p = 0$) and on the singularity of a closed dust-filled FRW Universe. He has taken the time variable from the matter variables, while Ryan's[12] time coordinate was Misner's[13] gravitational degree of freedom Ω. Ryan's method leads to a Klein–Gordon type equation for the wave function of the Universe, with its well known difficulties of interpretation while Lund's choice of time leads, in the cases he has considered, to a Schrödinger-like equation amenable to a probability interpretation (see ref. 20). By imposing the requirement that the corresponding mini-superspace[20–22] be geodesically complete, Lund concludes that, in this formalism, there is no inevitable singularity.

However, this Schrödinger equation, in the case of the closed FRW model, cannot be analytically solved and there is no guarantee that the wave function becomes zero for $R = 0$, which would be the case if it were impossible to find the Universe in the singular state. (For another possible definition of the absence of singularity in a quantum cosmological model, see ref. 23).

Thus, as far as we know, there has been no convincing evidence in the framework of quantum cosmology that the classical singularity could be avoided, under the influence of quantum gravitational fluctuations[24].

We give here an example of a non-singular quantum cosmological model, the 'dust-filled' Bianchi I Universe, "a behavioural paradigm for all anisotropic cosmological models"[25], quantised following the ADM method and with Lund's choice of time coordinate. The advantage of this model is that it can be analytically solved. As regards the hypothesis of a null pressure, it corresponds to the soft equation of state resulting from the Hagedorn[26] composite particle picture and it can also represent a cold, low-entropy early Universe filled with non-interacting baryons[27,28].

We use for the flat three-space theory of the Bianchi I Universe, Misner's[13] form:

$$\begin{aligned} \mathrm{d}l^2 = R_0^2\,\mathrm{e}^{2\mu}\{&\exp[2(\beta_+ + 3^{1/2}\beta_-)](\mathrm{d}x^1)^2 \\ &+ \exp[2(\beta_+ - 3^{1/2}\beta_-)]\mathrm{d}x^2)^2 \\ &+ \exp[-4\beta_+](\mathrm{d}x^3)^2\} \end{aligned} \tag{1}$$

R_0 is a constant and μ, β_+ and β_- are functions only of a time coordinate to be determined; μ, β_+ and β_- will be considered as the dynamical degrees of freedom.

We choose for the canonically conjugate moments to the g_{ij}, that is π^{ij}, the following expressions

$$\begin{aligned} \pi^{11} = &\left\{\frac{1}{12}[\pi_+ + 3^{1/2}\pi_-] + \frac{\pi_\mu}{6}\right\} \\ &\times R_0^{-2}\,\mathrm{e}^{-2\mu}\exp[-2(\beta_+ + 3^{1/2}\beta_-)] \end{aligned} \tag{2a}$$

$$\begin{aligned} \pi^{22} = &\left\{\frac{1}{12}[\pi_+ - 3^{1/2}\pi_-] + \frac{\pi_\mu}{6}\right\} \\ &\times R_0^{-2}\,\mathrm{e}^{-2\mu}\exp[-2(\beta_+ - 3^{1/2}\beta_-)] \end{aligned} \tag{2b}$$

$$\pi^{33} = \left\{-\frac{\pi_+}{6} + \frac{\pi_\mu}{6}\right\} R_0^{-2}\,\mathrm{e}^{-2\mu}\,\mathrm{e}^{4\beta_+} \tag{2c}$$

where π_μ, π_+ and π_- are the momenta conjugate to μ, β_+ and β_-, respectively. The action for the gravitational field and the matter (dust) can then be written as

$$\begin{aligned} S = \pi \int &[\pi_\mu\dot{\mu} + \pi_+\dot{\beta}_+ + \pi_-\dot{\beta}_- + \mathrm{p}^\phi\dot{\phi} - N(\mathscr{H}_\mathrm{V}^0 + \mathscr{H}_\mathrm{M}^0) \\ &- N_\mathrm{i}(\mathscr{H}_\mathrm{V}^\mathrm{i} + \mathscr{H}_\mathrm{M}^\mathrm{i})]\,\mathrm{d}t \end{aligned} \tag{3}$$

$\mathscr{H}_\mathrm{V}^0$ and $\mathscr{H}_\mathrm{M}^0$ are respectively the super-hamiltonian constraints for the vacuum[19] and for dust[16,19]

$$\mathscr{H}_\mathrm{V}^0 = \frac{R_0^{-3}\,\mathrm{e}^{-3\mu}}{24}[\pi_+^2 + \pi_-^2 - \pi_\mu^2] \tag{4a}$$

$$\mathscr{H}_\mathrm{M}^0 = 16\pi g^{1/2} N^2 (u^0)^2 \rho_0 = -N(u^0)p^\phi \tag{4b}$$

where ϕ is the only Seliger–Whitham velocity potential different from zero in this case; it is related to the four-velocity vector u_ν by

$$u_\nu = h^{-1}\frac{\partial\phi}{\partial x^\nu} \tag{5}$$

where h is the specific enthalpy of the fluid:

$$h = \frac{\rho + p}{\rho_0} \tag{6}$$

ρ_0 denoting the rest-mass density; p^ϕ is the momentum canonically conjugate to ϕ and N and N_i, respectively, the ADM lapse and shift functions; g denotes the determinant of the covariant spatial metric tensor and the overdots, in equation (3), the derivation with respect to the time variable, t.

Due to the homogeneity property of the t = constant surfaces of Bianchi-type universes, the supermomentum constraints: $\mathscr{H}_\mathrm{V}^\mathrm{i} + \mathscr{H}_\mathrm{M}^\mathrm{i} = 0$ are automatically satisfied.

Lund's method consists in choosing as time variable, the velocity potential ϕ:

$$T(t) = -\phi(t) \tag{7}$$

and imposing, in the framework of the ADM method, the coordinate condition:

$$t = T = -\phi \tag{8}$$

Then, solving the constraint: $\mathscr{H}_\mathrm{V}^0 + \mathscr{H}_\mathrm{M}^0 = 0$ for p^ϕ, we get for equation (3)

$$S = \pi \int [\pi_\mu\dot{\mu} + \pi_+\dot{\beta}_+ + \pi_-\dot{\beta}_- - H_\mathrm{ADM}]\,\mathrm{d}t \tag{9}$$

where the ADM hamiltonian, H_ADM is given by

$$H_\mathrm{ADM} = p^\phi = \frac{R_0^{-3}\,\mathrm{e}^{-3\mu}}{24}[\pi_+^2 + \pi_-^2 - \pi_\mu^2] \tag{10}$$

To quantise this model, we have to be careful: the usual choice of operators[14] corresponding to the classical conjugate momenta, for instance $\hat{\pi}_\mu = (1/i)(\partial/\partial\mu)$ is not appropriate here, as it would lead to a non-self-adjoint hamiltonian operator. However, if we introduce the canonical transformation

$$\begin{aligned} x &= 4(\tfrac{2}{3})^{1/2}\,\mathrm{e}^{3\mu/2} \\ \pi_x &= \tfrac{1}{4}(\tfrac{2}{3})^{1/2}\,\mathrm{e}^{3\mu/2}\pi_\mu \end{aligned} \tag{11}$$

the following hamiltonian operator

$$\hat{H}_{\rm ADM} = R_0^{-3}\frac{\partial^2}{\partial x^2} - \frac{4R_0^{-3}}{9x^2}\left[\frac{\partial^2}{\partial\beta_+^2} + \frac{\partial^2}{\partial\beta_-^2}\right] \qquad (12)$$

is self-adjoint with respect to the following inner product of wave functions of the Universe

$$\langle\Phi|\Psi\rangle = \iiint \Phi^*(x, \beta_+, \beta_-)\Psi(x, \beta_+, \beta_-)\,{\rm d}x\,{\rm d}\beta_+\,{\rm d}\beta_- \quad (13)$$

The Schrödinger equation to be solved for the wave function of the Bianchi I Universe is then

$$i\frac{\partial\Psi}{\partial t} = R_0^{-3}\left[\frac{\partial^2\Psi}{\partial x^2} - \frac{4}{9x^2}\left(\frac{\partial^2\Psi}{\partial\beta_+^2} + \frac{\partial^2\Psi}{\partial\beta_-^2}\right)\right] \qquad (14)$$

whose acceptable solutions for Ψ must be bounded in the complete domain of definition of the variable x, β_+ and β_-, that is

$$x \in [0, +\infty],\ \beta_+ \in [-\infty, +\infty] \text{ and } \beta_- \in [-\infty, +\infty].$$

The general solution Ψ of equation (14) can be written as a superimposition of particular solutions for the values E, p_+ and p_- of the separation constants

$$\Psi(x, \beta_+, \beta_-, t) = \alpha\iiint C(E, p_+, p_-)\Psi_{E,p_+,p_-}(x, \beta_+, \beta_-, t)\,{\rm d}E\,{\rm d}p_+\,{\rm d}p_- = \alpha\iiint C(E, p_+, p_-)\,{\rm e}^{-iEt}\cdot\exp[i(p_+\beta_+ + p_-\beta_-)]\cdot Y_{E,p_+,p_-}(x)\,{\rm d}E\,{\rm d}p_+\,{\rm d}p_- \qquad (15)$$

where $C(E, p_+, p_-)$ is the amplitude of the eigenstate of particular values E ($E < 0$; see equations (4b) and (10) see also Moncrief[30]), p_+ and p_- of the separation constants; α is an overall normalisation constant and $Y_{E,p_+,p_-}(x)$ is solution of

$$x^2\frac{{\rm d}^2Y}{{\rm d}x^2} + [\tfrac{4}{9}(p_+^2 + p_-^2) + R_0^3|E|x^2]Y = 0 \qquad (16)$$

This solution can be written as follows

$$Y_{E,p_+,p_-} = \bar{\alpha}x^{1/2}[AJ_{+(\frac{1}{4}-\frac{4}{9}(p_+^2+p_-^2))^{1/2}}(xC^{1/2}) + BJ_{-(\frac{1}{4}-\frac{4}{9}(p_+^2+p_-^2))^{1/2}}(xC^{1/2})] \qquad (17a)$$

With

$$C = |E|R_0^3 \qquad (17b)$$

Where J_ν is the Bessel function of order ν. A and B are constants determined by initial conditions and $\bar{\alpha}$ a constant of normalisation such that

$$\langle\Psi_{E,p_+,p_-}|\Psi_{E',p'_+,p'_-}\rangle = \delta(E-E')\delta(p_+-p'_+)\delta(p_--p'_-)$$

This solution is acceptable due to the asymptotic behaviour of $J_\nu(z)$ for $|z|\to\infty$. Moreover, by taking into account the behaviour of $J_\nu(z)$ for $z\to 0$, one deduces that

$$\Psi^*_{E,p_+,p_-}\Psi_{E,p_+,p_-} \xrightarrow[x\to 0]{} 0 \qquad (18)$$

We conclude that for a 'dust-filled' Bianchi I cosmological model, the classical singularity is avoided due to the presence of quantum gravitational effects in the sense that because $\Psi^*\Psi(x = 0) = 0$, it is impossible to find this Universe in the singular state.

For the isotropic version of the 'dust-filled' Bianchi I model, that is for the 'dust-filled' Einstein–de Sitter model ($\beta_+ = \beta_- = 0$), the wave function becomes zero at $x = 0$ only for a set of measure zero of models, which means that, in this case, the singularity will not be avoided.

The cause of possible singularity avoidance in the 'classical' quantum (or classical) regime[5–9], is usually ascribed to a violation of the energy condition of Hawking and Penrose's[1,2] theorems. We feel, however, that the non-singular character of the Bianchi I model studied here in the framework of quantum cosmology may be due to the fact that the quantum gravitational fluctuations do not allow the model to be sufficiently localised. Moreover, the singular Einstein–de Sitter models may be considered as very improbable (structurally unstable) models in the ensemble of all anisotropic Bianchi I Universes.

It would be interesting to extend the present work to the case of Bianchi-type models filled with a perfect fluid of equation of state: $p = (\gamma - 1)\rho$, which covers Zel'dovich's[30] stiff equation of state ($p = \rho$) as well as the hard equation ($p = \frac{1}{3}\rho$) characteristic of Collins and Perry's[31] 'quark soup'. This analysis would perhaps allow us to conclude as regards the truth of Wheeler's[32] conjecture "that every quantum solution for the problem of a closed universe within the framework of Einstein's field equations leads to a singularity". Work in this direction using Moncrief's[29] recent hamiltonian formalism for perfect fluids and Schutz's[17] formalism is in progress.

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Stability of quadruple junctions

THE analysis of triple junctions (points on the Earth where three lithospheric plates meet) has proved to be a powerful tool in deciphering the plate tectonic evolution of the Earth. In their classic paper on the evolution of triple junctions[1], McKenzie and Morgan dismissed the possibility of stable quadruple junctions existing more than instantaneously. We show here that quadruple junctions can be stable according to the vector velocity stability criteria of McKenzie and Morgan.

Consider the quadruple junction configuration of Fig. 1. Both spreading centres (A–B and C–D) extending into the junction

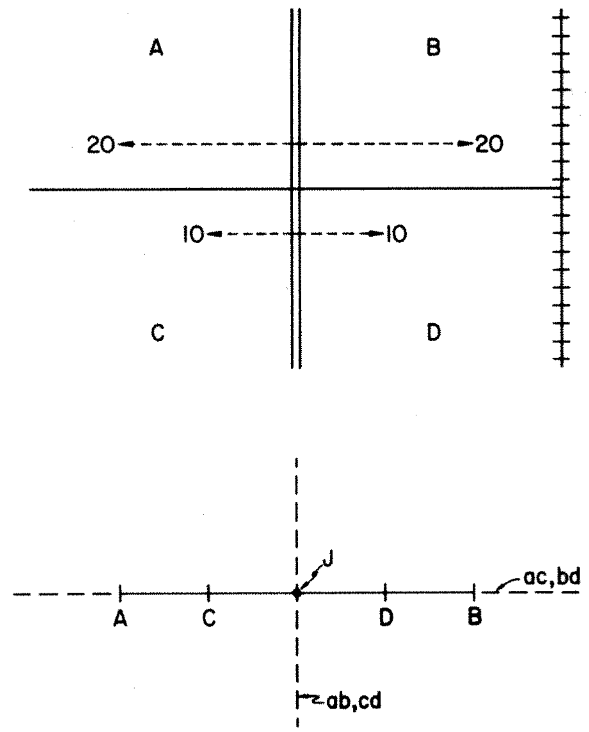

Fig. 1 Example of a stable quadruple junction and its representation in velocity space. Double lines are ridges, single lines are transform faults, hatchured line is trench. Arrows show relative plate motions. J represents quadruple junction.

spread symmetrically at the rates shown. Plate A therefore slips 10 mm yr^{-1} west relative to plate C and plate B slips 10 mm yr^{-1} east relative to plate D. This RFRF junction can clearly evolve continuously in this geometric configuration, which is confirmed by vector velocity analysis[1] (Fig. 1) or with scissors and paper. It is thus stable in the McKenzie–Morgan sense.

Many other stable quadruple junction configurations have been calculated by one of us (P.M.). Only the TFFF and FFFF junctions have no possible stable configuration. We note that stable quadruple and higher order *n*-tuple ridge junctions are possible if all *n* plate velocities fall along a circle in velocity space. All relative plate motions would be represented by chords of the circle, and the junction would have the vector velocity of the centre of the circle. Note that if this velocity criterion is met, the ridges can be at any orientations and the junction will be stable (the total angular spread of the ridges must be more than 180° if spreading is required to be non-oblique on all ridges; see ref. 3). If all four plate boundaries are orthogonal, five possible stable quadruple junctions may exist which do not require a specific ratio of plate velocities (RRRR, RFRF, RTRT, TTTF, and TTTT). In each of these cases, however, plate velocities are somewhat constrained by the geometric requirements; spreading is required to be symmetric and non-oblique, all transform faults are pure strike-slip, some subduction is required to be oblique and some non-oblique, and the subducted plate at each trench must be specified. These junctions are the only quadruple junctions which do not require both a specific geometry and specific plate velocities for stability. A possible situation in which a RRRR junction might be formed is during initial doming and rifting of a continent. If this happened, the subsequent establishment of normal seafloor spreading could result in a pair of failed rifts (aulocogenes) extending away from the quadruple junction.

There is a near quadruple junction today where the Pacific, Philippine, China and Australian plates nearly come together at the southern tip of the Philippine plate. There is also a near-juncture of the Pacific, Cocos, Rivera, and North America plates near the mouth of the Gulf of California. This could be a stable quadruple junction fixed with respect to the Rivera plate if the Pacific–Cocos spreading centre had the same vector velocity as the Rivera plate.

We agree with McKenzie and Morgan that quadruple junctions are unlikely to be of much importance in the Earth's evolutionary history, but a necessary step in identifying possible quadruple junctions is the recognition that they may exist.

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Charge transfer by surface states in the photoelectrolysis of water using a semiconductor electrode

THE photoelectrolysis of water using n-type semiconductors such as TiO$_2$, (refs 1–4) SrTiO$_3$, (refs 5–7) and so on is of current interest and importance for the utilisation of solar energy to produce hydrogen gas for fuel. In steady state conditions under illumination and in the absence of H$_2$ and O$_2$ in the electrolyte, the rate of H$_2$ evolution on a cathode is proportional to that of O$_2$ evolution on a semiconductor anode. The O$_2$ evolution process is considered to proceed by electron tunnelling from electrolyte levels to holes in the semiconductor through the Helmholtz layer, which must take place between states of equal energy. If only isoenergetic electron transfer between holes and occupied electrolyte states overlapping in energy the valence band occurs, the hole currents for O$_2$ evolution at n-TiO$_2$ would be expected to be very low, because the equilibrium redox level of the H$_2$O/O$_2$ couple is considerably above (at 1.8 eV for $pH = 4.7$ (ref. 2)) the top of the valence band. Experimentally, however, the current of holes at the TiO$_2$-electrolyte interface is found to be quite high and the efficiency in charge transfer of holes at the interface is almost 100%[8]. To account for such a high efficiency, a contribution from some surface states has been proposed[8,9]. A possible importance of surface states in electrochemistry at TiO$_2$ electrodes has been described by Frank and Bard[10]. Also, a recent electroluminescence measurement made for n-TiO$_2$ indicates that occupied surface states exist near the midgap at the TiO$_2$–electrolyte interface[11]. At present, however, it is not understood which kind or character of surface states can improve the charge transfer efficiency for holes at the interface. We present here a theoretical treatment of electron tunnelling between the semiconductor and electrolyte by surface states. We apply a theory of surface states (more exactly interface states) in tunnel MOS (metal-oxide–semiconductor) devices of Freeman and Dahlke[12] to the semiconductor–electrolyte interface. A quite high efficiency for holes at the interface can be obtained when the electron population of the surface states is controlled by the electrolyte and the surface states are almost completely occupied.

The energy band diagram of an n-type semiconductor in an electrolyte under hole injection (by illumination) is shown in Fig. 1. For simplicity we assume that the surface states can be described by a single energy level E_t with a surface density of N_t. The charging of the surface states can take place by interaction with electrons in the conduction and valence bands of the semiconductor and by tunnelling of electrons between the surface states and electrolyte. In the steady state condition, the total

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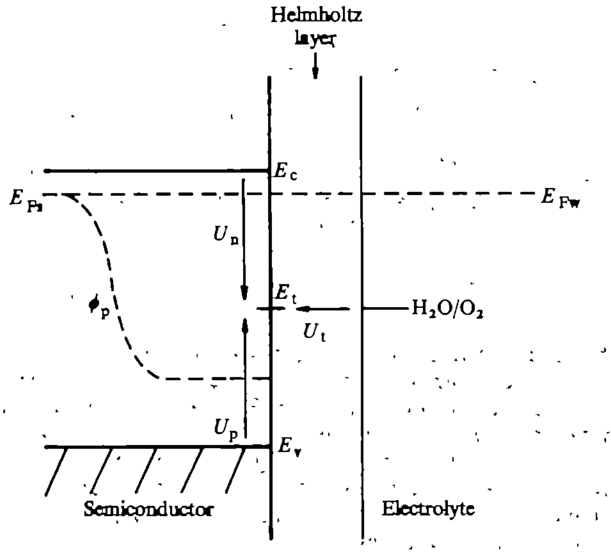

Fig. 1 Energy-level diagram for an n-type semiconductor–electrolyte interface with surface states at energy E_t and H_2O/O_2 redox level. The level E_{Fw} in the electrolyte and the Fermi level for electrons E_{Fs} in the semiconductor are aligned. The change in the quasi-Fermi level for holes ϕ_p is greatly exaggerated for clarity and indicates an increase in concentration of holes in the surface region of the semiconductor under illumination.

flux of electrons into the surface states must be equal zero. Then, the electron tunnel flux from the electrolyte into the surface states through the Helmholtz layer, which is proportional to the O_2 evolution rate, can be described as[12]

$$U_t = N_t(f_w - f_0)/(\tau_t + \tau_0) \tag{1}$$

Here, τ_t is a tunnelling time constant, f_w is an occupancy of the electrolyte states, and τ_0 and f_0 are constants characteristic of the surface recombination without tunnelling and are given by, respectively,

$$\tau_0 = [C_p(p_s + p_1) + C_n(n_s + n_1)]^{-1} \tag{2}$$

and

$$f_0 = \tau_0(C_p p_1 + C_n n_s) \tag{3}$$

where C_n and C_p are electron and hole capture coefficients, n_s and p_s are electron and hole concentrations at the surface of the semiconductor, and n_1 and p_1 are concentrations of electrons and holes to be expected when the Fermi level is at the surface state level.

If we assume that $\tau_t \ll \tau_0$ and $f_w = 1$, we have from equation (1)

$$U_t = N_t(C_p p_s + C_n n_1) \tag{4}$$

These conditions result in the situation that the electron population of the surface states is controlled by the electrolyte and the surface states are almost completely occupied. Furthermore, the condition of $\tau_t \ll \tau_0$ implies that the capture coefficients C_n and C_p in equation (2) should be sufficiently small.

Equation (4) indicates that U_t is determined by the hole capture from the valence band to the surface states and the electron emission from the surface states to the conduction band. In other words, an electron which tunnels from the electrolyte into the surface states either recombines with a hole in the valence band or enters the conduction band. The latter may be neglected compared with the former under illumination by which the surface concentration of holes p_s is increased, as n_1 is small when the surface state level is located near the midgap. Thus, U_t, that is the O_2 evolution rate is determined by the rate at which the almost completely occupied surface states capture holes at the semiconductor surface and is proportional to the surface concentration of holes, being in turn proportional to the light intensity of illumination, as observed experimentally. Because the hole emission from the surface states to the valence band is neglected in these conditions, it follows that all the holes reaching the semiconductor surface can be captured by the surface states and recombine with electrons in the electrolyte with the transfer probability of one. This can result in quite high efficiencies in charge transfer of holes at the interface.

Both the conditions of $\tau_t \ll \tau_0$ and $f_w = 1$ can be satisfied by the n-TiO_2 electrode, as the Helmholtz layer existing on the electrode is thin enough (of the order of 1 Å) to satisfy $\tau_t \ll \tau_0$ although the capture coefficients in TiO_2 are unknown, and $E_t = 1.5 \sim 1.24$ eV (ref. 11) and $E_{Fw} = 2.3$ eV (ref. 2) above the top of the valence band gives $f_w = 1$, where E_{Fw} corresponds to the Fermi level in the electrolyte. This explains the quite high transfer efficiencies observed at the TiO_2–electrolyte interface. But, it is not clear whether the surface states closely related to charge transfer at the TiO_2–electrolyte interface are pre-existing ones associated with surface $Ti^{3+}(3d^1)$ species[13,14] or some localised states occurring as a result of chemical bonding between the electrode and electrolyte. To clarify this point, further experiments for charge transfer at the interface using TiO_2 or $SrTiO_3$ electrodes with and without pre-existing surface states would be desired.

Finally, if we assume $\tau_t \gg \tau_0$, the U_t value is only a small fraction τ_0/τ_t of that for the case of $\tau_t \ll \tau_0$ and very low efficiencies in charge transfer are predicted.

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High frequency radar observations of horizontal plasma waves in the equatorial ionosphere

THE anomaly observed on the magnetic field records in equatorial zones is due to a strong ionospheric current flowing around 105 km, called the equatorial electrojet. We have used coherent radar returns from electron density irregularities embedded in this current to measure electric fields in this region and to study plasma instabilities at the origin of these irregularities. We report here that strong anomalous reversals of the equatorial electrojet are sometimes observed and quasi-horizontal plasma waves have been detected for the first time during such an event.

Plasma instabilities have been previously investigated in the equatorial electrojet using VHF and HF radars[1,2] and *in situ* measurements during rocket launches[3] While a good height resolution is obtained with rocket measurements, wave spectrum information has only been obtained through radar measurements. However, due to Earth curvature, the classical VHF radar, as used at 50 MHz in South America, is not adapted to detect plasma waves moving at grazing angles below about

10° at the electrojet altitude of 105 km (ref. 4). It is only when HF radars are used that the refraction in the E region of the lower frequency radio rays is sufficient for a possible detection of these horizontal plasma waves, the properties of which are known from the theory to be specially interesting[4].

Such an HF experiment has been developed near the magnetic equator at Addis Ababa (Ethiopia) since 1976 using a phase coherent HF radar operating at 7, 14, 21 and 29 MHz. At the lower frequencies, radio rays are usually totally reflected by the E layer during daytime but, while classical oblique radar echoes have been observed almost every day[2] and identified as the so-called 'type 1' or 'type 2' echoes discovered previously at VHF[1], nothing typical was ever detected related to horizontal radio rays. It is only during a very strong counter-electrojet event described elsewhere[5] that very anomalous echoes were found to be associated with the horizontal radio rays.

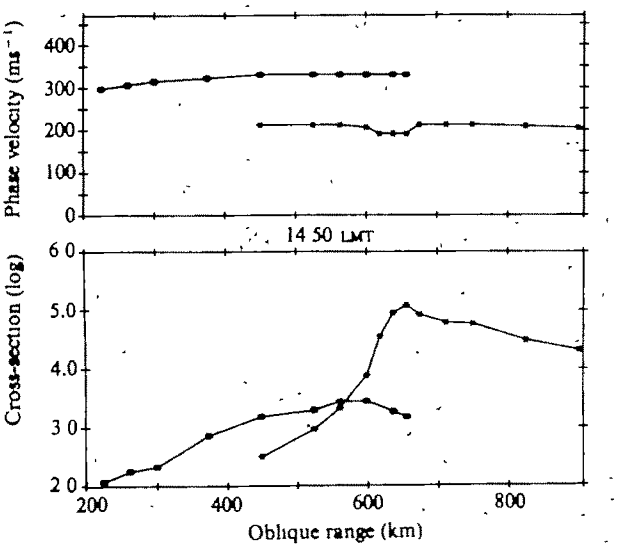
On 21 January 1977 coherent HF radar measurements were performed for the first time during a strong counter-electrojet. During such an event, the upward electron density gradient of the E layer is no longer destabilising due to the reversal of the electric field[6] and the cross-field instability cannot grow; however, the electron velocity is large enough (larger than the ion acoustic velocity in the medium) for the two stream instability to grow. It was then the first opportunity to investigate this instability without a background of turbulence due to the cross-field instability.

The spectral analysis of the radar echoes obtained at 14 MHz was performed at different time delays after the transmitted pulse corresponding to a variation of the zenith angle and a selection of different instability wave vectors (Fig. 1). The most striking and unexpected result is the discontinuity in both phase velocity and cross-section as a function of range and the simultaneous occurrence of two echoes for some ranges.

At the shortest ranges (or more oblique wave vectors) the phase velocity is larger and the cross-section much smaller than at the largest ranges (or more horizontal wave vectors). The shortest range echoes are similar to the usual 'type 1' echoes and are labelled accordingly (Fig. 1).

These experimental results can be considered in the light of a ray tracing of radio wave propagation for the conditions prevailing in the E layer at the time of the observations ($f_{oE} = 3$ MHz, $H_{max} = 105$ km). It is found (Fig. 2) that radio rays can be horizontal further than about 600 km, while strong refraction is still observed between 450 and 600 km. The strong amplitude and low phase velocity echoes labelled 'type H' on Fig. 1 can then be associated with horizontal and quasi-horizontal plasma wave vectors with a maximum cross-section for the horizontal waves. The association of the 'type H' echoes with the horizontal waves is confirmed by the time variation of the location of the amplitude maximum: the range of this maximum increases with time as given by the ray tracing when the E layer critical frequency decreases (that is, totally refracted echoes are observed at greater ranges).

Fig. 1 Phase velocity and scattering cross-section variation for type 1(O) and type H (•) echoes with the oblique range (in km) in the eastward direction at 14.50 LT on 21 January 1977 at Addis Ababa.



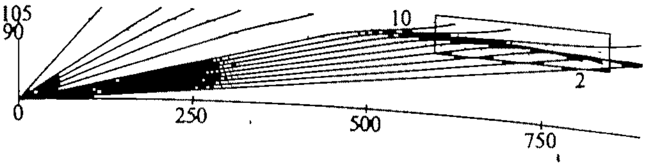


Fig. 2 Radio ray tracing at 14 MHz using a parabolic E layer ($f_{oE} = 3$ MHz, $h_{max} = 105$ km, $H = 7.5$ km) showing the horizontal radio rays further than 500 km.

The 'type H' echoes observed during the 21 January counter-electrojet event can then be associated with horizontal or quasi-horizontal plasma waves. The fact that such a phenomenon is not observed during normal electrojet conditions could be due to the turbulent state of the current flow when long wavelength cross-field instabilities grow in the normal daytime electrojet. When the electric field and the current are reversed during the counter-electrojet with no modification of the upward electron density gradient, the cross-field instability cannot grow and the turbulent mixing does not appear. The current flow is then always in a laminar state and the first waves appearing (the horizontal ones) will not have their growth rate modified by the turbulence as during normal electrojet conditions.

The characteristics of the 'type H' echoes cannot be definitely associated with either two stream (type 1) or cross-field (type 2) instabilities. On the one hand, the very narrow width of their spectrum, the strong amplitude and the lack of correlation between the phase velocity and the magnetic field variation are typical of type 1 spectra; on the other hand, the relatively low value of the phase velocity and the presence at the bottom of the layer are typical of type 2 echoes.

Among the two striking characteristics of type H echoes, the very strong amplitude is certainly associated with the fact that in a laminar electrojet the horizontal waves have the largest growth rate, but the other characteristic, the low value of the phase velocity, is still very puzzling and may be related either to some saturation effect similar to the quasi-linear reduction of the electric field[4] or to some secondary phenomena in the electrojet known to modify the phase velocity of the instabilities such as the presence of a strong neutral wind gradient with altitude or longitude or the occurrence of an anomalous gradient related to a thin blanketing sporadic E[7].

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Gradual increase of oceanic CO_2

SINCE 1850, human activities, mainly the burning of fossil fuels and the clearing of forests, have increased the amount of CO_2 in the atmosphere by ~50 p.p.m.[1-5]. Although it is generally believed that the oceanic CO_2 has increased correspondingly[6-11], only very limited data[12-14] have shown the increase quantitatively. We present here a computational calculation of the variation of the preformed total CO_2 (ΣCO_2^0) for a water mass, the original amount of total CO_2 (ΣCO_2) at the time of its last contact with the atmosphere. The result for the Atlantic Ocean reveals that the older water masses have invariably smaller ΣCO_2^0 as compared to the more recently formed waters.

Assuming a parcel of seawater in contact with the air remains at a constant degree of equilibrium[15] with the atmospheric CO_2, the amount of ΣCO_2 in this water is defined as ΣCO_2^0, which depends on the partial CO_2 pressure in the air (p_{CO_2}) as well as the salinity and the temperature of the seawater. Once the water sinks, the *in situ* decomposition of particulate $CaCO_3$ and organic carbon adds dissolved CO_2 to the parcel[16,17]. Consequently, the measured ΣCO_2 becomes larger than ΣCO_2^0. The values of ΣCO_2^0 can only be calculated from the measured ΣCO_2 values by correcting for the influences of $CaCO_3$ and organic carbon, which in turn can be calculated using the titration alkalinity (TA) and the apparent oxygen utilisation (AOU) data. (The combined effect of decomposing x moles of $CaCO_3$ and y moles of organic matter in 1 kg of seawater on ΣCO_2^0, TA^0 and AOU can be represented as[16,17]:

$$\Sigma CO_2 - \Sigma CO_2^0 = x + 106y$$

$$TA - TA^0 = 2x - 17y$$

$$AOU = 138y$$

Eliminating x and y from the above equations yields equation (1). Note that the constant associated with AOU is 0.83 instead of the often misused 0.768.)

$$\Sigma CO_2^0 = \Sigma CO_2\ (\text{measured}) - 0.5[TA(\text{measured}) - TA^0] - 0.83\ AOU \qquad (1)$$

where TA^0 is the preformed TA value. The values for TA^0 probably do not vary with the atmospheric CO_2 variation but depend only on the salinity and temperature. Therefore, we have assumed that the present day surface TA values represent the values for TA^0 during the past 130 yr.

The Geosecs (Geochemical Ocean Section Study) Atlantic surface TA data have been fitted to a function of potential temperature (θ)[18].

We have calculated TA^0 (and other chemical properties) on the constant salinity (35‰) basis. The values of chemical properties, for example TA^0, are normalised to 35‰ salinity (S) using:

$$TA^0 = \frac{TA^0(\text{measured}) \times S(\text{‰, measured})}{35.000}$$

$$\begin{aligned} TA^0(\mu\text{Equiv. kg}^{-1}) &= 2{,}368 - 4.72\theta, & \theta < 17\,^\circ C \\ &= 2{,}287, & \theta > 17\,^\circ C \end{aligned} \qquad (2)$$

Combining equations (1) and (2) gives:

$$\begin{aligned} \Sigma CO_2^0(\mu\text{mol kg}^{-1}) &= \Sigma CO_2(\text{measured}) - 0.5TA(\text{measured}) \\ &\quad - 0.83\ AOU + 1{,}184 - 2.36\theta, \quad \theta < 17\,^\circ C \qquad (3) \\ &= \Sigma CO_2(\text{measured}) - 0.5TA(\text{measured}) \\ &\quad - 0.83\ AOU + 1{,}143.5, \quad \theta > 17\,^\circ C \end{aligned}$$

The values for ΣCO_2^0 for the Atlantic waters formed in 1972 have been assumed to be equal to the surface ΣCO_2 values determined in that year (Geosecs). They are represented by

$$\Sigma CO_2^0(\mu\text{mol kg}^{-1}, 1972) = 2{,}195 - 9.5\theta \qquad (4)$$

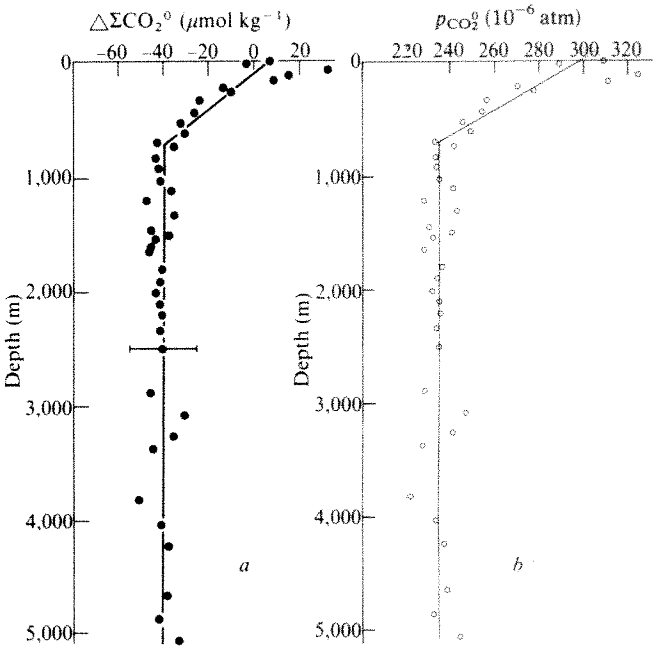

Fig. 1 Variations of $\Delta\Sigma CO_2^0$ (*a*) and $p_{CO_2^0}$ (*b*) with depth at 59°56.5′ S, 45°2.0′ W.

The difference in ΣCO_2^0 ($\Delta\Sigma CO_2^0$) between the older water masses calculated from equation (3) and the present day (that is 1972) values calculated from equation (4) is probably caused by changes in the atmospheric CO_2 concentrations. The uncertainty in $\Delta\Sigma CO_2^0$ is estimated to be ±15 μmol kg^{-1} due to the uncertainties in the measured chemical properties, namely ΣCO_2, TA and AOU.

The vertical variation of $\Delta\Sigma CO_2^0$ was calculated from a station in the Southern Ocean (Geosecs station 79; 59°56.5′ S, 45°2.0′ W). The values for $\Delta\Sigma CO_2^0$ decrease from approximately zero to nearly -40 ± 15 μmol kg^{-1} from the surface to 700 m, but remain constant from 700 m to 4,860 m (Fig. 1*a*). The decrease of roughly 40 ± 15 μmol kg^{-1} in $\Delta\Sigma CO_2^0$ with depth for the relatively young shallow waters reflects the decrease of atmospheric CO_2 with age. The deeper layer does not show depth-dependent variations in $\Delta\Sigma CO_2^0$, presumably because it was formed before the atmospheric CO_2 started to increase. The vertical profile for $p_{CO_2^0}$ (Fig. 1*b*) looks similar to the profile for $\Delta\Sigma CO_2^0$ and shows a decrease of 65 ± 30 p.p.m. from the surface to 700 m. Very little tritium, but large negative values for $\Delta^{14}C$ were found[19,20] below 600 m for waters south of 55° S, which confirms our conclusion. The variation in $p_{CO_2^0}$ is larger than the value generally accepted[1-5] or the value (50 p.p.m.) obtained by Brewer[14] using a similar approach.

Similar horizontal propagations of $\Delta\Sigma CO_2^0$ with latitude have been traced along the minimum salinity (Fig. 2*a*) and the minimum potential temperature (Fig. 2*b*) layers in the Atlantic Ocean. Figure 2*a* shows that the values for $\Delta\Sigma CO_2^0$ generally decrease from 45° to 20° S in the minimum salinity layer (~800 m deep). The break near 38° S is probably due to the change in flow patterns[21,22], as northwards flowing minimum salinity water turns eastwards at about 40° S and returns around the anticyclonic gyre. The water north of 38° S is significantly older than the water south of 38° S. There is probably another break at 22° S. The values of $\Delta\Sigma CO_2^0$ for water between 22° S and 25° N remain relatively constant at -40 ± 15 μmol kg^{-1}, indicating that there was no significant CO_2 variation in the atmosphere when this parcel of water was formed. The values

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for $\Delta\Sigma CO_2^0$ increase with latitude north of 25° N due to the influence of the freshly formed North Atlantic Deep Water[23]. There are no significant differences between the waters in the western and eastern Atlantic Ocean.

The tritium data support our findings. The average tritium value is 0.47 tritium unit (TU) at the depths of interest south of 38° S. This value is higher than the average value of 0.15 found between 38° and 22° S, which in turn, is higher than the average value of 0.06 TU for stations between 22° S and 25° N. Tritium concentrations are high for stations north of 25° N.

The values for $\Delta\Sigma CO_2^0$ in the minimum potential temperature layer (roughly 4,500 m deep) are shown in Fig. 2*b*. Waters in the eastern basin all have $\Delta\Sigma CO_2^0$ values of approximately -40 ± 15 μmol kg^{-1}, indicating that these waters formed before the industrialisation. Waters in the western basin are apparently younger. The value of $\Delta\Sigma CO_2^0$ is $\sim -20 \pm 15$ μmol kg^{-1} near 50° S in the western basin which suggests that the water here has been influenced by the atmospheric CO_2 increase. In the south, values for $\Delta\Sigma CO_2^0$ show a northwards decrease in the western basin. The values decrease to -40 ± 15 μmol kg^{-1} and remain constant from 38° S to 10° N, then start to increase north of 10° N, presumably due to the influence of the rapidly sinking North Atlantic Deep Water. The value reaches nearly zero at 47°33′ N at a depth of 4,546 m which indicates that the water here is probably not much more than a few years old. The distributions of $p_{CO_2^0}$ (not shown) look similar to that of $\Delta\Sigma CO_2^0$.

The computational scheme given above represents only first approximations of the very complicated systems in the oceans. The Redfield–Kethchum–Richards model[24] has been assumed to be valid everywhere in the oceans[25], and the possible effect of sulphur on TA[16,17] has not been considered. Waters with the same θ have been assumed to have the same TA^0 and ΣCO_2^0 and the complicated effects of mixing and the slow vertical diffusive migration of CO_2 from the surface water to deep water regime (T. Takahashi, personal communication) have been neglected. Nevertheless, the method is simple and the results reveal details that have not been shown before. [The work of Brewer was brought to our attention during the preparation of this letter. Although his computational scheme is different from ours, the concepts fall on the same line. His results (his Fig. 2) are similar to part of our Fig. 2*a*.] Quantitatively, the results show that the oceanic ΣCO_2^0 has increased by as much as 40 ± 15 μmol kg^{-1} (or 65 ± 30 p.p.m. in $p_{CO_2^0}$) responding to the atmospheric CO_2 increase. Qualitatively, the results have indicated how far and how deep the human induced CO_2 has penetrated into the Atlantic Ocean. We hope this method can help us to understand better the global carbon cycles.

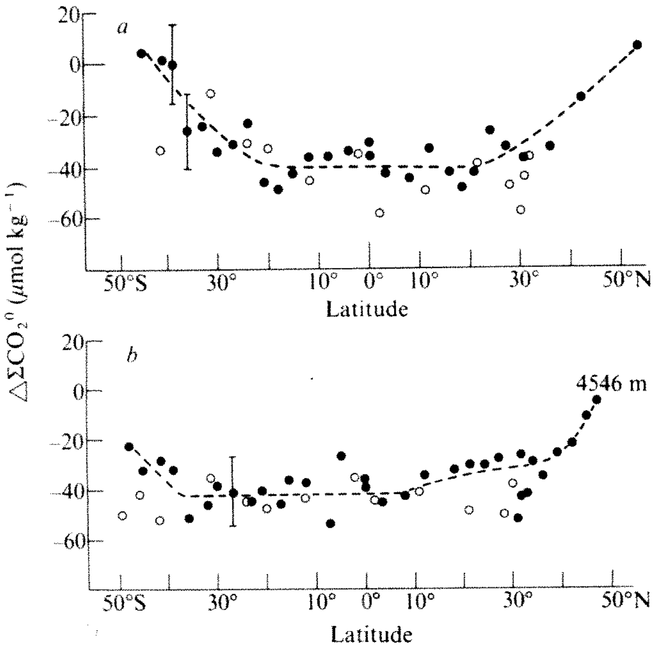

Fig. 2 Variation of $\Delta\Sigma CO_2^0$ with latitude along the minimum salinity (*a*) and minimum potential temperature (*b*) layers in the Atlantic Ocean. ●, Samples taken from the western basin; ○, samples from the eastern basin. The broken lines are drawn through the data in the western basin only.

Access to the Geosecs Atlantic data for alkalinity, ΣCO_2 and oxygen is acknowledged. We thank G. Ostlund, R. Fine, P. Niiler, L. Gordon and K. Johnson for assistance; C. Keeling, P. Brewer, T. Takahashi, R. Pytkowicz and E. Suess for helpful comments. The work was supported by the School of Oceanography, Oregon State University, and by grants from the Oceanography Branch of the NSF and the Office of Naval Research.

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Particulate uranium, plutonium and polonium in the biogeochemistries of the coastal zone

ALTHOUGH increasing attention has been paid to the role of inorganic solid phases in the chemistry of seawater, little quantitative data has been available to assess their involvement with living systems. We report here recent observations on the uptake of uranium, plutonium and polonium in coastal waters by organisms and submerged surfaces as traced by their isotopes. We show that the body burdens of these radioelements in some marine organisms are governed measurably by the uptake of their particulate forms. Furthermore, these elements are associated with different particulate phases as deduced from the rates at which they deposit on submerged surfaces.

The dissolved uranium content of marine waters seems to be related to the salinity[1]. For a salinity of 35‰, the uranium

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concentration is 3·35±0·02 μg l^{-1},(2.48 d.p.m. l^{-1}). Moreover, the ^{234}U/^{238}U activity ratio is reported as constant at 1.14±0.02 (ref. 2). The disequilibrium of the uranium isotopes in seawater is a consequence of the daughter ^{234}U entering a more readily dissolvable state than its parent, ^{238}U, following its production in crustal rocks. There are several explanations for this phenomenon[3]. However, the important observation is that the ^{234}U/^{238}U activity ratio in river water is usually greater than unity, while in the residual solids it is usually of the order of or less than unity. Thus, any continental solid phases that are mobilised to the ocean through the rivers, winds or glaciers may be identified by a ^{234}U/^{238}U ratio, whose value is <1. The distribution of plutonium and polonium between soluble and particulate phases in California coastal waters is unknown.

We began exposing solid surfaces (for example, glass beads) to seawater in 1976 to ascertain whether plutonium, which seemed to accumulate on the giant kelps in relation to surface area[4,5], would be collected by 'non-living' material. The solids were immersed in seawaters off the Scripps Institution of Oceanography Pier in La Jolla for periods of up to a month, and assayed for plutonium, polonium, and uranium[2,4]. Independent of the type of material, glass beads, filter paper, alumina balls or PVC, there is a direct relationship between exposure periods and the amounts of uranium, plutonium and polonium picked up from seawater per unit surface area (Fig. 1). Moreover, the uranium ratio of the sorbed phases varies between 0.91 and 1.07, averaging 0.98±0.03, with probable errors (counting errors) of 0.03 or less.

In the process of attempting to identify the possible organometallic complex of uranium, a batch of glass beads was leached with a mixture of chloroform and methanol (2:1). Little of the uranium, plutonium and polonium dissolved in solvent mixture and little was retained on the beads. Instead, these radioactive elements were found in the small amount of particulate material that was released from the beads by the solvent. These elements were probably on particles that were fixed to the beads by some type of natural organic adhesive. Thus, submerged bodies, including living algae, may, by an organic coating on their surfaces, collect some fraction of the particles, and the associated inorganic elements, that impact their surfaces in proportion to exposure time and surface area.

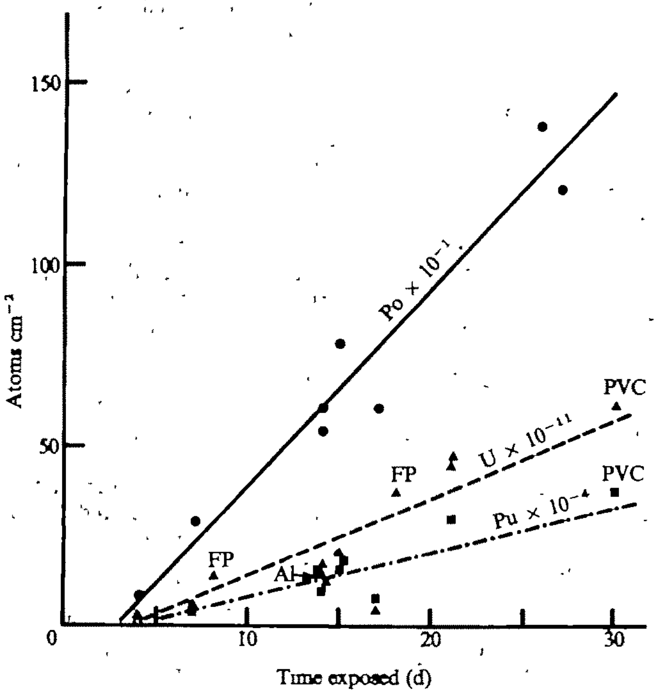

Fig. 1 Accumulations of uranium, plutonium and polonium in atoms cm^{-2} are plotted against the time exposed to seawater off the pier at Scripps Institution of Oceanography, except the 17-d exposure which is on a Navy platform one mile off shore. All points are for the exposure of glass beads except FP, Whatman 40 filter paper; Al, alumina balls; and PVC, polyvinyl chloride sheet. The accumulation rates are: U = 2.3×10^{11} atoms cm^{-2} d^{-1}; Pu = 1.2×10^{4} atoms cm^{-2} d^{-1}; and Po = 54 atoms cm^{-2} d^{-1}.

Table 1 ^{234}U/^{238}U activity ratio in particulates recovered from seawaters and in seawaters, from Southern California

Particulates collected					
By filtration (1 μm)	Date	^{234}U/^{238}U	^{238}U	$^{239+240}$Pu (d p m l^{-1})	^{210}Po
7,200 l	12/21/77	1 00±0.01	0.0022	0.000024	0.011
22,000 l	1/06/78	0 97±0 01	0 0023	0 000017	0 011
Collected by sedimentation and subsequently resuspended with overnight settling	11/10/77	1 00±0.02			
Collected by one month exposure of acrilan fibres	10/20/77	1.02±0.01			
Seawater					
Volume (l)					
unfiltered					
20	6/02/77	1.14±0 01			
20	7/22/77	1.15±0.01			
Dialysed					
20	9/01/77	1 16±0 01			
8	12/08/77	1.15±0.01			
8	12/19/77	1 15±0 01			

It is evident that a portion of uranium, plutonium and polonium in seawater is in particulate form. Of greater interest, however, is the difference in uptake slopes of all three elements on the solid surfaces (Fig. 1). This difference suggests that the elements, although in particulate form, may not be associated with the same solid phases.

Large volumes of seawater were pumped through 1 μm (nominal pore size) glass filters and the particulate material retained by the filters was found to contain concentrations of these elements in ratios roughly equal to those of the glass beads. The U/Po, U/Pu and Pu/Po ratios on an atom basis for the glass beads and seawater particulates were: 4.2×10^{9} and 2.4×10^{9}, 1.9×10^{7} and 2.1×10^{7}, 220 and 115 respectively. Moreover, the ^{234}U/^{238}U activity ratio of the particulates was unity (Table 1). Uranium isotopes were also determined on material that adhered to acrilan fibres exposed for one month in the kelp beds near Scripps and on sediment that collected in an 80 l barrel into which seawater flowed for a period of two months (Table 1). The barrel matter was resuspended in seawater and the fraction that did not settle overnight (<1–2 μm size fraction) was captured by centrifugation at 12,000g. The ^{234}U/^{238}U ratio of the particulates collected by these two methods was also essentially one (Table 1). In contrast, the total uranium in seawaters from the area has values of the ratio near 1.15 (Table 1). Volumes of seawater dialysed through membranes with molecular weight cut offs of 13,000 and 2,000 gave ^{234}U/^{238}U ratios of (1.15–1.16)±0.01 in the dialysate.

Thus, coastal seawaters near San Diego, California, possess a ^{234}U/^{238}U activity ratio of 1.15±0.01, while the particulate phases contained therein and originating on the continents have values around unity. Even though the particulate materials comprise <1% of the uranium content in the dissolved state, could their impact on the biosphere be quantified? To assess this possibility, the uranium in the soft tissues of filter feeding organisms was assayed for their uranium isotopic composition (Table 2). Brown algae were also analysed.

For most of the biological samples, the ^{234}U/^{238}U activity ratio is less than that of dissolved uranium (1.15). Note that the soluble uranium activities contributed by the water that constitutes the difference between the wet and dry weights were not subtracted and would only tend to enhance the role of particulate uranium if a correction were made. The largest deviation from the seawater is in scallops with a value of 0.96±0.01. On the basis of the uranium isotopic ratio, assuming uranium of dissolved origin has a ratio of 1.15 and particulate uranium a ratio of 0.98, all the uranium found in the scallops was due to the uptake of particulate uranium. Likewise, the observation that the mussels have an average value for the ratio of 1.13 indicates that about 11% of their uranium was taken up as solid phases. The mussels have remarkably high values of uranium in their byssal threads, over 10 times more than in their bodies on the average, and this uranium seems to have arisen primarily from the dissolved state in seawater.

The tunicates, on the other hand, have values of the $^{234}U/^{238}U$ ratio markedly lower than those of the mussels. These filter feeders have high hydrogen ion concentrations in their gut regions which could leach uranium from the particulate phases. Both bodies and gut were analysed in three samples. In every case the gut materials had lower values of the ratio but similar values of the uranium concentrations compared to the bodies. Clearly, the particulate phases in seawater contribute significantly to the uranium body burden of these organisms.

The $^{234}U/^{238}U$ activity ratio in the algae ranges from 1.09 to 1.16, indicating contributions from both dissolved and particulate uranium. The elk kelp blade (1.16) was thick relative to the macrocystis blades, and thus had less surface area per unit weight for particle deposition. The reverse is seen with the elk kelp scrapings, which maximise the area to weight and have the lowest ratio of the uranium isotopes (1.09)—indicating that 35% of the accumulated uranium was in the particulate form.

Polonium and plutonium are most probably taken up by the algae in a particulate state, by impact as opposed to active processes. The Pu/Po atom ratios on the glass beads and kelp are 220 and 280 respectively. The ratio for seawater particulates is 115, also roughly the same. Even more remarkable is that the rates of uptake of polonium and plutonium in atoms cm^{-2} d^{-1} by the glass beads and the kelp are essentially identical, Po = 54 and 52 (ref. 4) and Pu = 1.2×10^4 for both the beads and kelp[4].

Table 2 Uranium concentrations and isotopic composition in marine organisms from California coast (on a dry weight basis)

		^{238}U (d.p.m per kg)	$^{234}U/^{238}U$ activity ratio
Algae			
Elk kelp (large blade)		102±1	1.16±0.02
Elk kelp (surface scrapings)		—	1.09±0.02
Macrocystis		358±4	1.13±0.01
Macrocystis		508±2	1.10±0.01
Mussels (*Mytilus* sp.)			
Pt Fermin		130±1	1.12±0.01
Pt Arguello		118±1	1.13±0.01
Bodega Head		121±1	1.13±0.01
Oceanside		224±1	1.09±0.01
San Simeon		138±1	1.12±0.01
Diablo Canyon		200±1	1.15±0.01
Santa Cruz		177±1	1.11±0.01
Soberanas Pt		241±1	1.15±0.01
N San Francisco		251±1	1.15±0.01
S San Francisco		172±1	1.10±0.01
Farallon Is		246±1	1.15±0.01
San Diego		125±1	1.13±0.01
Byssal threads			
Bodega Head		9,100±40	1.12±0.01
Cape Flattery		3,860±30	1.15±0.02
Farallon Islands		4,640±20	1.13±0.01
Tunicates			
Polyclinum planum		140±1	1.07±0.01
		63±1	1.12±0.01
Styela montereensis	body–1	116±1	1.10±0.02
	gut–1	149±1	1.03±0.02
	body–2	207±1	1.06±0.01
	gut–2	245±1	1.03±0.01
Ciona intestinalis	body	245±1	1.09±0.02
	gut	330±1	0.98±0.02
Abalone		760±1	1.13±0.01
Gooseneck barnacle		206±1	1.07±0.01
Barnacle gills+intestine		169±1	1.13±0.02
Scallops		329±1	0.96±0.01
Rock oysters		278±1	1.07±0.05

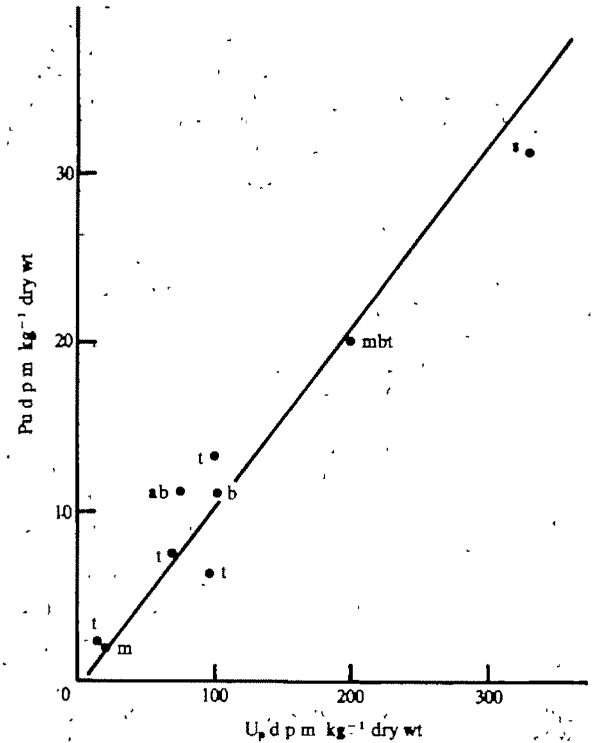

Fig. 2 The activity of plutonium and particulate uranium U_p are linearly proportional with a slope of 1/10. s, scallops; mbt, mussel byssal threads; t, tunicates; ab, abalones; b, barnacles; and m, mussels.

The plutonium concentrations in the other marine organisms are linearly proportional to the uranium of particulate origin (Fig. 2). Thus, the uptake of plutonium by these organisms may be related to the ingestion of this transuranic in a particulate form.

Clearly, uptake of particulates can significantly influence the uranium, polonium and plutonium content and the uranium isotopic ratio of coastal marine organisms. By simply measuring the uranium isotopic ratios, the extent to which particulate uranium is important can be quantitatively determined. Using the same approach, it may be possible to assess the role of particulate trace elements by comparing their specific activities, for example, ^{210}Pb/stable lead or $^{238}Pu/^{239+240}Pu$ on particulate phases in seawater and in the organisms.

These studies also point out that caution must be exercised in calculating a concentration factor for trace elements in organisms over the concentration of that element in seawater. For example, the scallops accumulate particulate uranium exclusively and, therefore, using the ^{238}U particulate concentration in seawater of 0.002 d.p.m. l^{-1} (Table 1), the concentration factor based on the dry weight of the organisms is 1.5×10^5. Had the total ^{238}U seawater concentration been used to calculate this factor, a value of roughly 150 would have been reported. Note that the build up on the beads after one month of exposure to seawater for uranium, plutonium and polonium is ~1×10^7, using the fact that 4.5 kg of beads collect nearly 1 g of dry particulates. On the other hand, the byssal threads of the mussels from Bodega Head, California, have a concentration factor for soluble uranium of 3.3×10^3 and one for insoluble uranium of 7.4×10^5.

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^{10}Be in marine sediments, Earth's environment and cosmic rays

COSMIC RAYS create the long-lived radioactive nuclide ^{10}Be (half life: 1.5 M yr) continuously in the upper atmosphere as a result of nuclear disintegrations. It enters the oceans through various paths and finally deposits in deep-sea sediment in a time which is short compared to its half life. This isotope has been used to establish the chronology of geophysical events up to the Pliocene[1–7] or to suggest cosmic-ray intensity variations in the past[8,9]. This report shows that the ^{10}Be stratigraphy in marine sediments can be used as a time marker for geophysical events in the past, but not for delineating any time variations in cosmic-ray intensity unless one can learn how to delineate geophysical effects. Also the cosmic-ray consistency would be estimated from the limits of ^{10}Be variation implied by this data set.

A sediment core (KH68-4-18), 968 cm in length, was collected from a 5,390 m deep-ocean basin at 01° 59′ N, 170° 01′ W, central equatorial Pacific. Beryllium was separated from the sediment samples using an improved chemical method[10], and the β-ray activity of ^{10}Be was counted with two sets of low-level needle counters[11] and a low-level silicon detector; background count rates and counting efficiencies of these were $0.0082 \pm 0.0005\ \mathrm{min}^{-1}$, $51 \pm 3\%$, $0.0072 \pm 0.0005\ \mathrm{min}^{-1}$, $51 \pm 3\%$, $0.0372 \pm 0.012\ \mathrm{min}^{-1}$, $35 \pm 2\%$ (90–555 keV), respectively.

The absolute age $T(x)$ at a depth x was evaluated[12] from a rate of ^{10}Be participation, P (atoms cm^{-2} s^{-1}), onto sediment surface and the specific activities of ^{10}Be, $N(x)$ (d.p.m. cm^{-3} or atoms cm^{-3}), in all successive layers along the core down to a depth, x.

$$N(x)=\frac{P}{S(x)}\exp\left[-\lambda T(x)\right]. \qquad dT(x)=\frac{dx}{S(x)}$$

or

$$T(x)=-\frac{1}{\lambda}\ln\left[1-\frac{\lambda}{P}\int_0^x N(x)\,dx\right]$$

Here, λ is a decay constant of ^{10}Be and P is assumed to be constant and equal to 2.15×10^{-2} ^{10}Be atoms cm^{-2} s^{-1}, during the time interval considered for this core, and the sedimentation rate at a layer, $S(x)$, is assumed to have varied with depth or time.

The specific activities of ^{10}Be and the ^{10}Be ages are listed in Table 1 together with magnetic ages[13], differential sedimentation rates and the content of Be and Mn. The uncertainty in the specific activities was estimated on the basis of the uncertainties (1σ) in chemical determination of beryllium (8%), counting efficiency (5%), absorption correction (5%) and counting statistics (7–17%).

The ^{10}Be ages agree well with the magnetic ages at six reversal horizons (Table 1). The magnetic reversal pattern of core KH68-4-18 is very clear and consistent with palaeontological analysis[13]. These agreements show that the assumption of constant ^{10}Be precipitation would be correct and the cosmic-ray production rates integrated over the time intervals ($2–7 \times 10^5$ yr) between each adjacent reversals have been constant ($< \pm 10\%$

Table 1 Specific activities of ^{10}Be, ^{10}Be ages, magnetic ages, differential sedimentation rates and concentration of chemical elements in core KH68-4-18

Depth interval (cm)	Dry weight of sample (g)	*In situ* density* (g cm^{-3})	Specific activity of ^{10}Be (10^{-3} d.p.m. cm^{-3})	^{10}Be age (Myr)	Magnetic age† (Myr)	Differential sedimentation rate (mm kyr^{-1})	Chemical elements Be (p.p.m.)	Chemical elements Mn (%)
0– 35	9.7	0.24	1.77 ± 0.23	0.11		3.2	1.85	0.55
35– 75	14.2	0.24	1.57 ± 0.20	0.22		3.6	1.78	0.53
75–110	12 4	0.25	1.20 ± 0.17	0.30		4.4	1.65	0.32
110–155	13.7	0.24	1.25 ± 0.15	0.41		4.1	1 66	0.55
155–190	12.1	0.25	1.25 ± 0.18	0.50		3.9	2.24	0 56
190–230	13.8	0.24	0.97 ± 0.13	0.59		4.4	1.49	0.54
230–265	11.6	0.24	1.37 ± 0.20	0.69	0.69	3.5	1.88	0.56
265–300	10.0	0.24	1.09 ± 0.15	0.78		3.9	1.75	0.60
300–335	11.7	0.23	1.05 ± 0.15	0.87	0.89	3.9	2.15	0.46
335–365	9.8	0.23	1.19 ± 0.20	0.97	0.95	3.0	1.52	0.44
365–415	18.8	0.25	1.21 ± 0.17	1.13		3.1	1.72	0.56
415–465	18.9	0.26	0.62 ± 0.09	1.23		5.0	1.70	0.66
465–515	17.9	0.24	1.34 ± 0.18	1.43		2.5	1.82	0.64
515–570	18 3	0.24	1.01 ± 0.13	1.61	1.65	3.1	1.76	0.61
570–625	12.0	0.23	1.14 ± 0.16	1.85	1.85	2.3	1.64	0.51
625–675	16.7	0.22	0.76 ± 0.10	2.00		3.3	1.79	0.52
675–730	17.2	0.22	0.75 ± 0.12	2.19		2.9	1.87	0.50
730–790	22.5	0.24	0.69 ± 0.13	2.39		3.0	1.70	0.44
790–850	20.1	0.23	0.14 ± 0.08	2.43	2.43	15.0	1.56	0.37
850–905	18 6	0.23	0.34 ± 0.06	2.53		5.5	1.59	0.53
905–968	18.5	0.23	0.86 ± 0.16	2.84		2.0	1.53	0.46

* *In situ* density is defined as the ratio of the dry weight of sediment to the *in situ* volume occupied by the sediment.

† The depth is measured from the top of clay sediment filled in core pipe, whereas the depth presented in ref. 5 where the magnetic age has been determined is measured from the top of core pipe; the difference is 15 cm. The magnetic age tabulated here is normalised to the present depth.

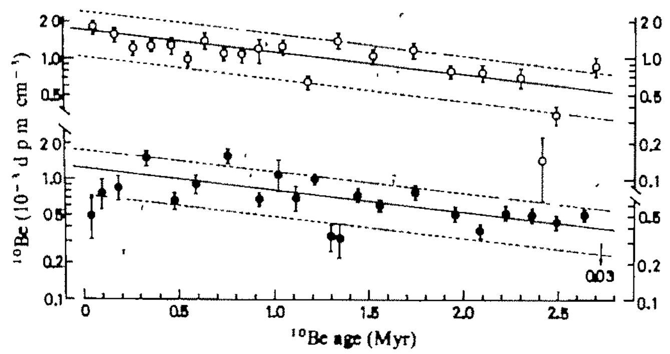

Fig. 1 Comparison of the ^{10}Be variations with time in two Pacific deep-sea sediment cores: ○, KH68-4-18; ●, KH68-4-25. Data for core KH68-4-25 taken from ref. 12. Mean decay lines are shown by solid lines, with ±40% limits of variation by dotted lines.

variation) up to 2.5 Myr BP. The differential sedimentation rates at different layers vary by a factor of 2 from ~2.5 mm kyr^{-1} to ~5 mm kyr^{-1} except for 15 mm kyr^{-1} at a layer 790–850 cm. The mean sedimentation rate is 3.5 mm kyr^{-1} consistent with the value of 3.4 mm kyr^{-1} (ref. 13) derived from magnetic stratigraphy.

The ^{10}Be activities in core KH68-4-18 (2° N, 170° W) and a previously investigated[12] core KH68-4-25 (20° S, 170° W) are plotted against the ^{10}Be ages in Fig. 1.

The ^{10}Be activities in the two cores do not parallel each other in the past. This fact rules out cosmic-ray intensity changes as the principal cause for the ^{10}Be variations. Another fact unfavourable to the simple cosmic-ray interpretation is that the rate of ^{10}Be precipitation at 2° N (2.15×10^{-2} atoms cm^{-2} s^{-1}) is twice as large as that at 20° S (1.14×10^{-2} atoms cm^{-2} s^{-1}, see ref. 12). If the ^{10}Be precipitation rate reflects the latitudinal variation of the overhead ^{10}Be fallout[14], the P values for the two cores would be reversed.

Non-parallelism of the ^{10}Be variations in the two cores is also unfavourable to a simple global climate change as a main cause for the ^{10}Be variations[15], although the glacial and interglacial effects in principle could influence the ^{10}Be content of ocean sediments in a relatively complex fashion.

One of the most significant differences between the two cores is the low ^{10}Be concentrations (Recent 0.27 Myr) at the top of core KH68-4-25, that do not occur for core KH68-4-18. Tanaka *et al.*[12,16] showed that the volcanism of Samoan island arc caused the decrease at the top of core KH68-4-25, on the bases of the abundance of volcanic glassy fragments and the variation of elemental abundances. In core KH68-4-18, there are no glassy fragments of volcanic origin and no correlation between the concentrations of ^{10}Be and elemental abundances; volcanism has little effect for this core. This equatorial core has plenty of fossils of faunal and floral species throughout (30–80% in volume under microscope), with exceptions at depth 415–465 cm (1.13–1.23 Myr) and 790–850 cm (2.39–2.43 Myr) where the fossil occurrences are sporadic (less than a few %) and the ^{10}Be concentrations are low (see Fig. 1). Biological productivity may cause changes in ^{10}Be concentrations.

Another set of analysis will be stated on the limits of ^{10}Be variation implied by this data set. The data show some scatter in the plot of log $N(x)$ versus time (Fig. 1), but the decay line (solid lines in Fig. 1) having the slope of a half life of 1.5 Myr which starts from the $N(0)$ value derived from the adopted P value and mean sedimentation rate seems to be representative for the data set. The limits of ^{10}Be variation from the mean line are ±40% for both cores, represented by dotted lines in Fig. 1. A few points beyond the limits can be explained by special effects such as the presence of volcanic fragments or the low biological productivity. The ^{10}Be variations are mainly affected by complex environmental factors, and the cosmic-ray intensity variations, if any, do not exceed at most ±40% for period of ~1×10^5 yr (sampling intervals) during the past 2.5 Myr.

The ^{10}Be variations with time and with oceanic location reflect complex changes in the Earth's environment such as volcanism, biological productivity, oceanic circulation, and climate. The cosmic-ray intensity variations, if any, do not exceed ±10% for periods of 2–~7×10^5 yr and ±40% for periods of ~1×10^5 yr during the past 2.5 Myr, and can not be delineated from this data set.

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Human chimaera detectable only by investigation of her progeny

A CHIMAERA can be defined as an organism whose cells derive from two or more zygotes[1]. In man, two categories of spontaneous and permanent chimaeras are recognised: blood chimaeras which arise from the exchange of blood-forming cells between dizygotic twins, and dispermic chimaeras which are the result of the fusion of two zygotes and of their growth into one body (for review see ref. 2). Blood chimaeras are usually identified because of blood-grouping problems caused by the mixture of different erythrocyte populations, while dispermic chimaeras who often present sex abnormalities are mainly detected by cytogenetic studies, although they also carry a mixture of different blood cell populations. In contrast to these typical cases, we now report a unique human chimaera who shows no mixture of blood cells as far as the genetic markers investigated are concerned, and who is only detectable by the investigation of the blood of her progeny (family Eiw., Fig. 1).

This chimaera was brought to our attention because of an apparent mother–child exclusion in the ABO system: mother II.2: A_2B, child III.4: 0. Blood samples of the mother II.2, her husband II.1, her children III.1–III.4 and her parents I.1 and I.2 were typed for the following markers: ABO, rhesus, MNSs, P, Kell, Duffy, Kidd, Lutheran, Lewis, ABH- and Lewis-secretion, Xg, acid phosphatase acP-1, phosphoglucomutase PGM-1 (including the subtypes, PGM-1*, defined by isoelectric focusing on polyacrylamide gel[3]), adenylate kinase AK-1, adenosine desaminase ADA, glutamate pyruvate transaminase GPT, glyoxalase GLO-1, esterase D EsD, gammaglobulin groups Gm

Table 1 Genetic marker systems showing mother–child exclusions (family Eiw.)

	Born	ABO	Rhesus	Kidd	AcP-1	PGM-1^a	Bf	
I.1	1896	B	CcDee	a+b+	B	NT*	S	A1, 2; B
I.2	1901	A_2	CcDee	a+b+	AB	a3–a1	FS	A3, w31;
II.1	1924	B	ccddee	a+b+	B	a1	S	A3, 26; B1
II.2	1937	A_2B	ccddee	a−b+	B.	a3–a2	S	A2, w31; B7,
III.1	1967	B	ccddee	a+b−†	B	a2–a1	FS‡	A3, 26; Bw35,
III.2	1969	O†	ccddee	a+b+	AB‡	a1†	FS‡	A3, 26; Bw35,
III.3	1970	B	CcDee‡	a−b+	AB‡	a1†	FS‡	A3, B15, w35; C
III.4	1975	O†	CcDee‡	a−b+	B	a1†	FS‡	A3, 26; Bw35, w38

* PGM-1^a not tested, PGM-1 phenotype of I.1 in starch gel electrophoresis: 2-1.
† Mother–child exclusions without taking into account the father II.1.
‡ Mother–child exclusions taking into account the father II.1.

and Km, haptoglobins Hp, group specific components Gc, properdin factor B Bf and HLA.

It became evident that all four children III.1–III.4 did not fit genetically with their mother II.2 whilst there was no evidence for an exclusion of the father II.1. The systems showing mother–child exclusions without taking into account the father II.2 are listed in Table 1: III.1 is excluded by Kidd and HLA, III.2 and III.4 by ABO, PGM-1^a and HLA and III.3 by PGM-1^a and HLA. Accepting the paternity of II.1, the mother is additionally excluded from III.1–III.4 by Bf, from III.2 and III.3 by acP-1 and from III.3 and III.4 by rhesus (Table 1). Thus, the mother–child exclusions include genes governed by chromosome 1 (rhesus and PGM-1^a)[4,5], chromosome 2 (acP-1)[6], chromosome 6 (Bf and HLA)[7,8], chromosome 9 (ABO)[9], and possibly chromosome 7 (Kidd)[10]. Significantly, all the genes (or haplotypes) which must have been transmitted from the mother II.1 to her four children and which are not detectable in her blood can be found in the maternal grandparents: *O* potentially present in I.1 and in I.2, *CDe* and *Jk^a* in I.1 and I.2, acP-1 *P^a*, *Bf^F* and *HLA-A3*, *Bw35*, *Cw4* in I.2, and PGM-1^a *a*^{1} in I.2 and possibly in I.1. The four children III.1–III.4 are therefore genetically compatible with their father II.1 on the one hand and their maternal grandparents I.1 and I.2 on the other hand.

The results of unidirectional mixed lymphocyte cultures (Table 2) showed that I.2 is able to stimulate her daughter's (II.2) lymphocytes, although II.2 carries obviously in her organism both HLA haplotypes of her mother. The stimulation observed is in good agreement with a one HLA haplotype difference between both individuals. This lack of immunological tolerance demonstrates that all the cells of II.2 which express HLA-D gene products (lymphocytes, monocytes[11], epithelial[12] and endothelial cells[13]) do not possess the HLA haplotype *A3*, *Bw35*, *Cw4*.

Fibroblasts of II.2 have been cultivated from skin biopsies of both forearms. The typing results of all the markers tested for in these cells (acP-1, PGM-1^a, EsD, GLO-1 and HLA) were identical to the findings obtained using blood cells.

The karyotypes of all family members were analysed using Q-, G- and C-banding techniques. There was no evidence for numerical or structural abnormalities and, checking chromosome polymorphisms, no mother–child exclusion could be found. The mother II.2, in particular, showed in her lymphocytes and in her fibroblasts the normal female karyotype 46, XX.

Table 2 Results of unidirectional mixed lymphocyte cultures (MLC) in family Eiw. expressed in c.p.m. of ^{3}H-thymidine uptake

	Stimulators (X-irradiated)		
Responders	I.2$_X$	II.2$_X$	UR$_X$*
I.2	716	17,154	60,279
II.2	9,200	1,319	26,007
UR*	55,783	33,825	951

The numbers represent the mean value of ^{3}H-thymidine uptake in triplicate tests.
* Unrelated control person.

An interchange of all four children, *a priori* improbable, co be ruled out by interrogating the staff of the obstetric department in which the four children had been born. Furthermore the mother II.2 has no twin sibling and does not show in her circulation more than one erythrocyte and/or lymphocyte population.

The findings in this family clearly show that II.2 possesses in her organism at least two populations of genetically different cells, one demonstrable in her blood as well as in the fibroblasts, and the other one in her gonads; therefore, she must be considered a chimaera. Although the analysis of the genetic markers carried by the different cell populations of II.2 gives no evidence for the involvement of two sperms, the most simple explanation of this observation is to assume a dispermic chimaerism. The cells with the HLA haplotype *A3*, *Bw35*, *Cw4* present in the gonads of II.2 must have a rather restricted tissue distribution, as the cells of ectodermal and mesodermal origin studied do not carry the HLA-D determinant coded for by this haplotype.

Thus this family demonstrates the existence of chimaeras who do not show different populations of erythrocytes, lymphocytes and fibroblasts and can be detected only by the analysis of their progeny. Such individuals must be extremely rare, but the practical implications of this observation for problems of parentage are clear.

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progeny populations were bred from
ed-X method[3]. This yield stocks of flies in
have identical, mutagenised X chromosomes.
each stock were selected under CO_2 anaesthesia and
ed for testing 1–3 days later. To screen for mutants with abbreviated memory retention, we trained flies from each mutagenised population by exposing them 10 times to a chemical odorant, 3-octanol, in association with shock[5], then left them undisturbed for 45 min to give them an opportunity to forget. The flies were tested by running them alternately towards (1) a tube containing 3-octanol (O), the odorant they had been taught to avoid, and (2) a tube containing 4-methylcyclohexanol (M) a novel, control odorant. Figure 1 shows the number of flies which avoided each. To control for odour bias, flies which had been trained to avoid M were tested in the same apparatus. The index of learning, Λ, is the fraction of the population avoiding the shock-associated odour, minus the fraction avoiding the control odour, averaged for the two halves of the experiment. (All Λ values reported here are mean ± s.e.m. for 10 experiments.) In these conditions, wild-type flies learn and retain selective avoidance for 45 min. Seven hundred mutagenised stocks were tested; one, *amnesiac* (*amn*^{P1801}) gave reproducibly poor performance in this paradigm.

The *amnesiac* males selected above were used to breed a pure stock of mutant males and females. Male *amn* flies were mated to females carrying the X-chromosome balancer FM7a (ref. 6)

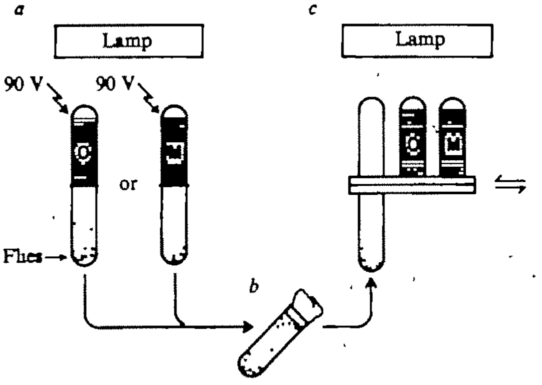

Fig. 1 Measuring memory retention of mutagenised stocks. *a*, Training: a population of 30–50 flies was taught to avoid a specific odour. The light attracted the flies from the start tube into a contiguous tube containing a chemical odorant (either 1 μl 3-octanol (O) or 2 μl 4-methylcyclohexanol (M), spread on an electrified grid (60 Hz, 90 V a.c.). After 60 s, the flies were shaken back into the start tube and another training run was begun. This cycle was repeated for 10 runs. *b*, Waiting: flies were left undisturbed for 45 min in dim light at room temperature (22 °C). *c*, Testing: the sliding counter-current apparatus[1] allowed the fresh start tube, with flies, to be shifted opposite either of two (non-electrified) grids, one spread with O, the other with M. The flies were shifted opposite the O grid and given 15 s to run, after which the flies remaining in the start tube were counted. After 45 s rest in the 'rest' tube (without grid), they were shifted opposite the M tube and their avoidance was similarly scored.

Table 1 Latent memory in normal and *amnesiac* flies

	Λ_1 (flies trained twice to same odour)	Λ_2 (flies trained to opposite odours)	$\Lambda_1 - \Lambda_2$
No anaesthesia			
C-S	0.24 ± 0.02	0.01 ± 0.03	0.23 ± 0.04
amnesiac	0.28 ± 0.03	0.03 ± 0.01	0.24 ± 0.04
Anaesthesia 30 min after first training session			
C-S	0.20 ± 0.03	0.07 ± 0.01	0.13 ± 0.03
amnesiac	0.17 ± 0.02	0.04 ± 0.01	0.13 ± 0.03

Populations were trained 10 times to avoid O or M as in Fig. 1, then left undisturbed for 45 min. Flies were then given a single cycle of training[1], which consisted of a 30-s run towards an odour (O or M) with shock, a 30-s wait, and a 30-s run towards the control odour (M or O) without shock. Thirty seconds later the flies were tested as usual. The four possible combinations of two aversive training sessions were run 10 times each. Scores from flies trained against the same odour in both sessions (O → O and M → M) were averaged to give Λ_1 values. Similarly, scores from flies trained against opposite odours (O → M and M → O) give Λ_2 values. Learning indices are calculated relative to the last training session; positive difference between the two Λ values indicates that the earlier training session affected behaviour. Such a difference is present for both normal and *amnesiac* flies. Moreover, in both genotypes, the difference persists when the flies are briefly anaesthetised 30 min after the first training sessions. Therefore cryptic memory, present in *amnesiac* flies, can be consolidated to the long-term (anaesthesia-resistant) form.

and C-S autosomes. Female FM7a/*amn* progeny were mated to *amnesiac* males, yielding F_2 progeny from which a pure stock of hemizygous *amn* males and homozygous *amn*/*amn* females could be selected by the absence of *Bar*, a dominant visible marker on the FM7a chromosome. All subsequent experiments on *amnesiac* were done with mixed males and females, 3–5 days old.

The 10-trial training procedure above is a modification of the original learning paradigm to give enhanced memory retention. It was used only for mutant screening and in genetic mapping and latent memory experiments as specified below. All other memory measurements were made using the basic olfactory paradigm of ref. 1. This is similar to the procedure in Fig. 1 except that it uses 3 training trials instead of 10, and during training, flies are alternately exposed to both O and M (one with shock, one without), to provide an internal control for sensitisation. In this olfactory paradigm the expression of learning is statistical; even in our best conditions only a third of the flies show learning in any given trial. This limits our understanding of what individual flies in the population are doing. However, we have previously found that normal C-S flies appear to be homogeneous in their capability to learn; the selective avoidance observed is not due to an 'intelligent' subset of the population[1]. A likely reason for our relatively low learning scores is that the task we have chosen—olfactory discrimination and active avoidance—is a difficult one for *Drosophila*. In fact, the flies learn much better in a simpler paradigm, as that originally developed for cockroaches by Horridge[8]. Of 25 normal individuals tested 24 (96%) consistently lifted their legs to avoid shock (data not shown).

Populations of *amnesiac* and normal C-S flies were trained in the olfactory paradigm, left undisturbed for various intervals, then tested to obtain the memory decay curves in Fig. 2. Immediate learning in the two stocks is similar, but memory in *amnesiac* flies decays rapidly and is undetectable 60 min later (avoidance of both shock-associated and control odours decreases, so that 60 min after training the behaviour of *amnesiac* is undistinguishable from that of untrained flies). In contrast, memory in C-S persists for 6 hours in these conditions[2].

Some uninteresting explanations for *amnesiac*'s forgetfulness have been ruled out. The decline in the mutant's performance is not due merely to lethargy at testing time. Populations were

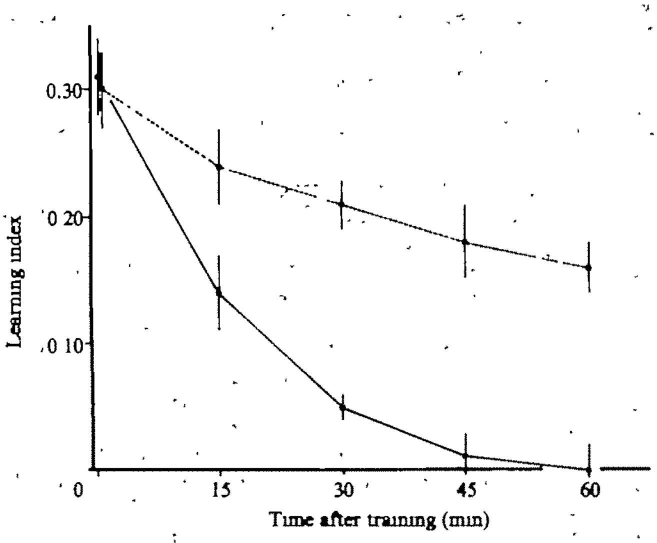

Fig. 2 Memory retention of normal (dashed line) and *amnesiac* (solid line) flies. Populations of C-S and *amnesiac* flies were given three training trials, left undisturbed for the periods indicated, then tested (procedure is as in ref. 1, except that flies were shifted to fresh tubes just before testing). The two genotypes were trained and tested simultaneously

trained, left for 45 min, then given brief (30-s) electric shock, in an attempt to re-invigourate them, and tested. The mutant still failed to demonstrate memory [$\Lambda(amn) = 0.03 \pm 0.03$, $\Lambda(\text{C-S}) = 0.15 \pm 0.02$]. To find out whether the memory decline is a result of a general deterioration in *amnesiac*'s capabilities brought on by trauma in training, flies were trained, left for 60 minutes, trained again (half to the same odour, half to the other) and immediately tested. The mutant still had normal capacity for learning ($\Lambda = 0.32 \pm 0.02$).

The sensory and motor abilities of *amnesiac* appear normal. Phototaxis as measured by countercurrent distribution[7] is similar to C-S. Mutant flies show normal avoidance of electrified grids, jump when shocked, and have normal leg-to-leg resistance of about $10^9\ \Omega$ (ref. 3). The sensitivity of *amnesiac* to the odour cues used is also similar to the parent strain. Normal flies have a slight intrinsic tendency to avoid the odorants O and M, which is overcome in the learning paradigm by strong phototactic drive toward the odour tubes. This aversion can be used to measure the flies' olfactory capability in a 'choice chamber'—a T-maze in which flies are passively transported to the choice point. One arm contains a defined amount of odorant; the other is left blank[3]. In this test, the odour avoidance behaviour of normal and *amnesiac* flies was indistinguishable. The fraction of the population which avoided O (2 μl per tube) was 0.83 ± 0.03 for *amnesiac*, and 0.81 ± 0.04 for C-S. The fraction avoiding M (2 μl per tube) was 0.65 ± 0.06 for *amnesiac*, and 0.70 ± 0.03 for C-S.

Table 2 Mapping the *amnesiac* mutation

Class	Memory (Λ) 45 min after training
Parental chromosomes:	
amnesiac	0.04 ± 0.01 ($n = 14$)
sc ec cv ct^6 *v g*^{2} *f*	0.19 ± 0.03 ($n = 27$)
Recombinant chromosomes:	
ec cv ct^6 *v g*^{2} *f*	0.21 ± 0.06 ($n = 9$)
cv ct^6 *v g*^{2} *f*	0.15 ± 0.02 ($n = 9$)
ct^6 *v g*^{2} *f*	0.10 ± 0.01 ($n = 8$)
v g^2 *f*	0.14 ± 0.01 ($n = 16$)
g^2 *f*	0.15 ± 0.03 ($n = 14$)
f	0.17 ± 0.02 ($n = 17$)
sc	-0.01 ± 0.03 ($n = 10$)
sc ec	0.06 ± 0.02 ($n = 12$)
sc ec cv	0.10 ± 0.02 ($n = 8$)
sc ec cv ct^6	0.04 ± 0.03 ($n = 9$)
sc ec cv ct^6 *v*	0.03 ± 0.03 ($n = 10$)
sc ec cv ct^6 *v g*^{2}	0.05 ± 0.03 ($n = 9$)

Memory scores, 45 min after training, are shown for parental chromosomes and each recombinant class. Each chromosome was tested over *amnesiac* in heterozygous females, to eliminate effects of markers. Recombinant chromosomes with the *forked* marker have high memory scores; chromosomes with *forked*$^+$ have low scores. This places the location of the *amnesiac* marker very close or to the right of *forked*, near the centromere of the X chromosome.

We also measured *amnesiac*'s memory using other odour cues. Flies were trained and tested as in Fig. 2, except that amylacetate and benzaldehyde[2] (both 2 μl per grid) were substituted for O and M. As before, the mutants learned normally ($\Lambda(amn) = 0.31$, $\Lambda(\text{C-S}) = 0.30 \pm 0.01$) but forgot quickly; 45 min after training $\Lambda(amn) = 0.02 \pm 0.01$, while $\Lambda(\text{C-S}) = 0.15 \pm 0.02$.

Drosophila, like higher animals, has separate memory storage phases—short-term memory, which is sensitive to cold anaesthesia, and long-term memory, which is not[2,5]. It seemed possible that the *amnesiac* mutation acted by blocking consolidation into long-term memory. Testing this idea directly is impossible, because memory in the mutant is virtually undetectable by the time consolidation would normally take place. Therefore, to lengthen *amnesiac*'s memory span, we increased the number of training trials and adopted a procedure akin to the psychological technique of 'savings measurement', in which memory can interact with more recent learning.

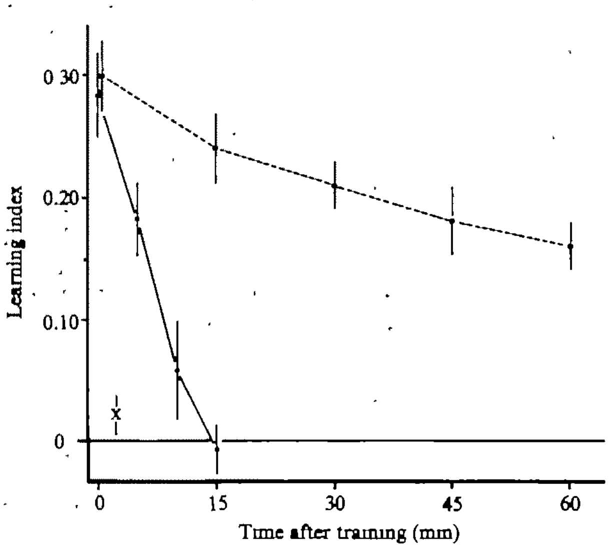

Fig. 3 Memory retention in normal flies (dashed line), *turnip* mutants (X), and *turnip*/+ heterozygotes (solid line). Method as in Fig. 2. The heterozygotes did not regain their memory later; learning indices at 20 and 60 minutes remained near zero.

We shocked flies 10 times in conjunction with either O or M, left them undisturbed for 45 min to forget, then gave them a single training run to one of the odours and tested them. All four possible combinations of the two training sessions were run, that is: O → O, M → M, O → M and M → O. If latent memory from the first training session were present to interact with learning from the final training cycle, populations which had been trained to avoid the same odour in both sessions (O → O and M → M) should show higher learning scores than populations trained in opposite directions (O → M and M → O). Table 1 shows that this was the case, for both normal and *amnesiac* flies. Moreover, for both genotypes, the effect of the first training session was largely resistant to brief cold anaesthesia applied 30 min later. The *amnesiac* mutants, like normal flies, can convert their memory into anaesthesia-resistant form; so the mutation does not block consolidation into long-term memory.

More surprisingly, Table 1 shows that in these conditions the earlier training has quantitatively similar behavioural effects in normal and *amnesiac* flies. Apparently, memory is present in the mutant. It is cryptic after the first training session, inaccessible to direct expression ($\Lambda = 0.02 \pm 0.02$) but can interact with more recent learning to affect behaviour just as in normal flies. This suggests that the *amnesiac* mutation affects memory retrieval rather than storage.

Genetically, *amnesiac* behaves as a sex-linked recessive. Memory of *amn*/+ heterozygotes 45 min after training was normal ($\Lambda = 0.18 \pm 0.03$). The mutation was mapped on the X chromosome using methods similar to those of Dudai *et al*[3]. Female *amnesiac* flies were crossed with males carrying the visible X-linked markers *scute*, *echinus*, *cut*^{6}, *vermilion*, *garnet*^{2}, *forked*. Recombinant F$_2$ males were collected and scored for the visible markers. These could not be tested directly for the presence of *amnesiac* because the markers affect the performance in learning tests. Therefore, recombinants were individually mated to *amnesiac* females, producing female progeny in which the recessive visible markers were heterozygous, and not expressed. These F$_3$ females are homozygous for *amn* if the mutation was present in the F$_2$ male; if the females forget rapidly one can infer that *amn* was present in the original recombinant. Table 2 shows memory scores of progeny from the various recombinant types. It can be seen that the *amnesiac* mutation maps near or to the right of *forked* (1–56.7) on the X chromosome. Preliminary data from a second cross suggest that *amn* lies between *forked* and *carnation* (1–62.5).

We originally set out to isolate mutants affected in memory (in contrast to those blocked in immediate learning) because memory mutations should act in the central nervous system. The fact that *amnesiac* learns in the first place indicates that its sensory and motor systems are functioning properly. Moreover, the action of memory mutations should be relatively well defined. To learn, a fly must sense cues and reinforcement, transmit the information to the proper neural centre, encode the association, and later convert it to appropriate motor output. This is a series of complex processes; a mutation could affect any of them. In contrast, memory retention requires two things—that information be stored in a relatively permanent form and that it be accessible to retrieval. We believe the *amnesiac* mutation affects one of these, probably accessibility to retrieval. Further understanding of the mutation's action on memory will depend on finding anatomical or biochemical abnormalities in *amnesiac* flies.

Gaining substantive information about memory retention will probably require more than one mutant. It is worth noting that other mutations can affect memory. Y. Dudai (personal communication) has evidence that *dunce* flies[3] store learned information in a labile form for a very short time after training. We have isolated a mutant, *turnip*(*tur*^{PS274}) which is apparently blocked in immediate learning. The heterozygote, *turnip*/+ learns normally but forgets very rapidly (Fig. 3). A plausible explanation for this situation is that the homozygote, more severely affected in retention, forgets immediately after training so that no learning can be detected.

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Interactions between *Entamoeba histolytica* and complement

THE pathogenic mechanisms in amoebiasis are not fully understood. Histopathological studies of infected tissues have shown lysis of host cells near the parasites, particularly in the liver where abscesses are formed. In the intestine, inflammatory reactions are often slight, unless there is secondary bacterial involvement. The infiltrating cells belong mainly to the lymphocyte and macrophage cell series, and neutrophil leukocytes are sparse[1], suggesting that there is a low-grade chronic inflammation. Tacheuchi and Phillips[2] have made systematic studies of experimentally infected germ-free guinea pigs, which have revealed the early events in invasive amoebiasis. When the amoebae had not yet made contact with the colonic epithelial cells, the latter showed degenerative changes such as loss of brush borders, swelling of mitochondria and dilatation of the endoplasmic reticulum. At the same time polymorphonuclear leukocytes (PMN) as well as some macrophages were seen to infiltrate the lamina propria, and extravasated erythrocytes were observed in the same areas. A high proportion of the PMN showed signs of degeneration, including disruption of the cell membrane. As changes were observed some distance from the parasites, Tacheuchi and Phillips[2] concluded that the parasites exert their effects by means of a soluble mediator(s), possibly a toxin elaborated by the parasites. Another possibility is that enzymes released from amoebae damage both epithelium and PMN. A third explanation would be that the amoebae can interact with host material to generate factors which are chemotactic for leukocytes and can also exert toxic effects. A likely candidate would be the complement system. For this reason, the experiments described here were undertaken to analyse interactions between amoebae and the complement system. IgG antibody fractions were prepared from sera of individuals suffering from amoebiasis, and unheated sera from normal humans or experimental animals were used as a source of heat-labile complement components. We found that *Entamoeba histolytica* activates complement by the alternative pathway, and can be lysed by the reaction products.

Table 1 % Of amoebae lysed by human sera in various experimental conditions

Serum	Unheated	Heated	Unheated +EDTA	Unheated +EGTA	Unheated −properdin
BF	37	2	2	43	5
GH	46	2	5	50	2
BH	42	3	1	51	NT
IC	39	1	3	44	NT
PH	43	2	6	46	NT
Normal rabbit	NT	3	NT	NT	NT

Hk 9 strain of *E. histolytica*, cultivated axenically. Normal human sera, lacking demonstrable antibodies, were diluted 1 : 3 without heating or after heating at 56 °C for 30 min; EDTA or EGTA were used in final concentration of 5 mM. Assays were carried out in cytotoxicity trays (Møller-Coates), using 6 μl containing about 200 amoebae. Assays were carried out in quadruplicate using 0.1% trypan blue, counting a total number of 400–500 cells. Sera were depleted of properdin by affinity chromatography on an anti-properdin-activated Sepharose column at 4 °C in the presence of 10 mM EDTA (ref. 14) which was later removed by dialysis in the cold. After this procedure the serum was still capable of lysing amoebae in the presence of antibodies.

Two strains of *E. histolytica*, Hk 9 and NIH 200, were grown in culture. When amoebae from either one of these strains was incubated in fresh human serum without detectable antibodies, a high proportion of the amoebae were lysed, as shown by marked morphological changes, including disruption of the cell membrane and expulsion of nuclei. These results were

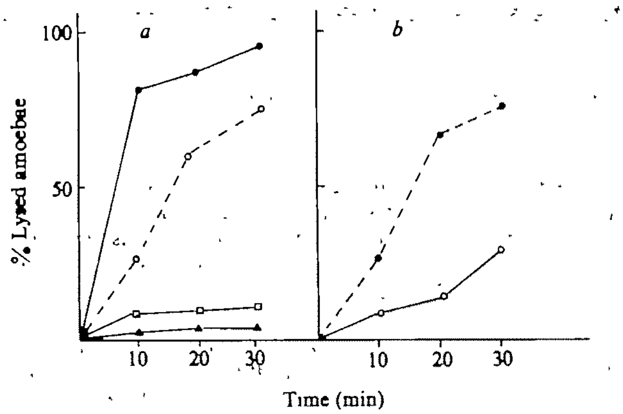

Fig. 1 % Dead amoebae (Hk 9) after incubation for 10, 20 and 30 min with unheated or heated normal human serum diluted 1:2 in phosphate-buffered saline, pH 7.4, containing Ca^{2+} and Mg^{2+} (PBS) *a*, ●, Amoebae preincubated for 30 min at 4 °C with the IgG fraction of a human serum containing antibodies to *E. histolytica*. The fraction was obtained by ion-exchange chromatography on a DEAE Sephadex A50 column. Immunofluorescence with class-specific antiglobulin conjugates showed an IgG titre of 40, IgM and IgA negative. After interaction with IgG antibodies, fresh unheated human serum with no detectable antibodies was added to give a final dilution of 1:2. ○, Amoebae preincubated at 4 °C for 30 min in PBS followed by reaction with unheated serum. □, Preincubation with IgG antibodies followed by heated serum. △, Preincubation in PBS followed by heated serum. *b*, ○, Preincubation with IgG antibodies followed by unheated serum + 5 mM EGTA. ●, Preincubation without antibodies followed by unheated serum + 5 mM EGTA.

confirmed by the release from prelabelled cells into the medium of either ^3H-uridine or ^{14}C-α-isobutyric acid. However, the use of isotopes was somewhat less sensitive, as well as less convenient, than morphological observations, and for most experiments the latter were used. The lysis was inhibited by 5 mM EDTA, which chelates both Ca^{2+} and Mg^{2+} ions, but not by 5 mM EGTA, which selectively chelates Ca^{2+} ions. When sera were depleted of properdin, lysis did not occur (Table 1). Lysis was efficient in the presence of $\geqslant$50% normal guinea pig serum as well as C4-deficient guinea pig serum, in which there is a complete block of the classical pathway (Table 2).

Two distinct effects of antibodies were demonstrated. If amoebae were incubated with the IgG fraction of an antiserum and then with unheated serum, accelerated lysis was observed, presumably due to classical pathway activation. However, the lysis occurring in the presence of EGTA was markedly inhibited (Fig. 1). This may be due to steric hindrance by antibodies of access to groups on the amoeba surface with alternative-pathway activating properties.

Immunofluorescence showed the presence of C3 or a derivative with the same immunological reactivity on the surface of the amoebae. Capping followed by endocytosis occurred in a similar way to that described by Aust Kettis and Sundqvist[3] in their studies of the capping of specific antibodies on the surface of *E. histolytica*.

These observations demonstrate that *E. histolytica* is able to activate complement by the alternative pathway and that the parasites can be lysed by the reaction products. Furthermore, sera from rabbits congenitally deficient in C6 gave markedly reduced lysis compared to sera from normal rabbits, and depletion of normal human serum of C5 by affinity chromatography abolished its capacity to lyse amoebae. These findings indicate that lysis is due to the terminal complement components (C5–C9).

Serum dilutions higher than 1:10 resulted in very little lysis. Experiments were designed to ascertain whether such sub-lytic concentrations of complement might stimulate amoebae to increased secretion of hydrolytic enzymes in a manner analogous to the secretion-stimulating effects of immune complexes[4] and isolated C3b (ref. 5) on macrophages. In repeated experiments it was found that both the total concentration and release of the acid hydrolase *N*-acetyl-β-D-glucosaminidase (NAGase) were increased in amoebae which were grown in the presence of 5–10% unheated serum, in contrast to comparable amounts of heated serum (Fig. 2).

The relevance of these observations to the pathogenesis of amoebiasis is not yet known. Activated complement may participate in defence against amoebae. Nevertheless, despite the lytic effect of complement, *E. histolytica* can in many instances survive in the tissues. Continuous turnover of parasite cell membrane components accompanied by change in distribution of complement receptors may contribute to their survival in the host. Moreover, in the intestinal wall the concentration of active complement components may be at the limit of what is required to lyse the parasite. In this situation complement cleavage products, such as C3b, might stimulate the amoebae to increased production and release of hydrolytic enzymes. Such enzymes

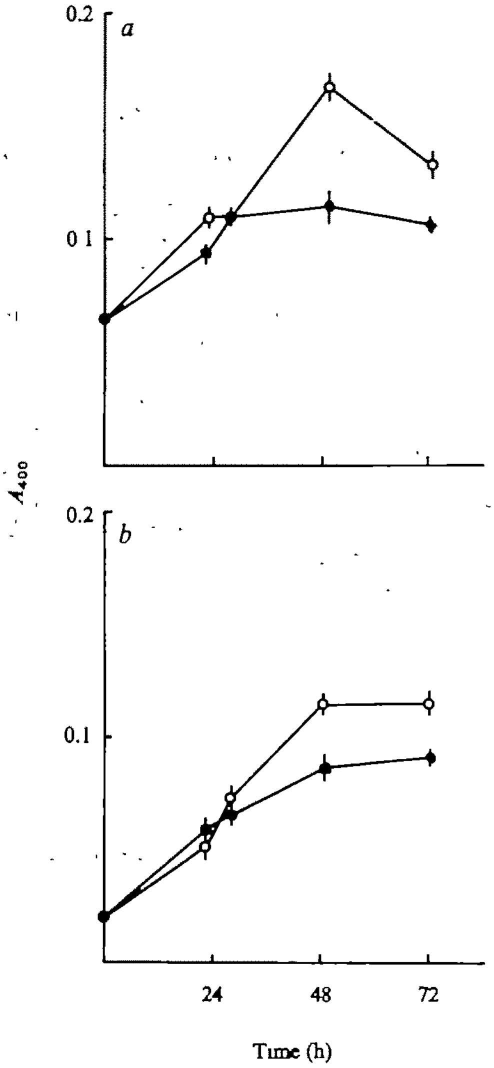

Fig. 2 *a*, Total amount of NAGase per 1000 amoebae; *b*, amount of enzyme released into the medium per 1000 amoebae. *E. histolytica* (NIH 200) was cultured in 1.8-ml glass tubes with screw caps (PBG-Trident) in medium TPS-1 with the addition of either 10% heat-inactivated or 5% heat-inactivated and 5% unheated human serum. At 22, 28, 49 and 72 h four tubes in each series were chilled in an ice bath, shaken in a whirl mixer and 1 ml removed for parasite counting in a Coulter Counter Industrial B. The remainder were centrifuged for 5 min at 1,500 r.p.m. Enzyme assay according to Woollen *et al.*[15] was carried out on supernatants and cells. ●, 10% heated human serum; ○, 5% heated + unheated normal serum.

seem to be involved in the pathogenesis of many chronic inflammatory conditions[6], and the combined effects of activated complement, leukocyte products and amoebic enzymes probably contribute to the lytic and reactive changes observed in the tissues of patients with invasive amoebiasis.

Table 2 % Of amoeba lysed by normal and C4-deficient unheated and heated guinea pig serum in various experimental conditions

Serum	% Dead parasite cells
Unheated	57
Unheated + EDTA	2
Unheated + EGTA	75
Heated	5
C4 deficient, unheated	54
C4 deficient, heated	3

E. histolytica strain NIH 200 was incubated with 3:4 dilutions of guinea pig sera. Other experimental conditions as for Table 1.

Capacity to activate complement by the alternative pathway has also been reported for other parasites, including *Trypanosoma cruzi*[7,8], African trypanosomes, including the variant-specific antigen[9–11], and *Schistosoma mansoni*[12]. Schistosomula incubated with normal serum activate complement by the alternative pathway, and the C3 on the surface of the parasites allows attachment of eosinophils and killing of the parasites[13]. Hence, activation of complement by the alternative pathway can not only lyse parasites directly, as described here for enteropathic amoebae, but also render parasites susceptible to complement-dependent cell-mediated lysis. Complement activation, presumably initiated by surface glycoproteins of parasites, may be a relatively common phenomenon, contributing on the one hand to host protection and on the other hand to the pathogenesis of parasitic diseases.

Sera from rabbits congenitally deficient in C6 were obtained from Professor K. Rother, goat anti-human C5 serum was provided by Dr D. R. Schultz, strains of *E. histolytica* by Dr L. Diamond, absorbed rabbit anti-properdin serum by Dr C. O. Kindmark, and C4-deficient guinea pig serum by Dr P. Lachmann.

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HLA and testicular cancer

HLA IS involved not only in transplantation, but is also associated with many disease states. This first became evident when the major histocompatibility complex of the mouse (H-2) was shown to play a major part in determining resistance to Gross leukaemia virus[1]. Subsequent studies in both animals and man have shown that many disease states are associated with, and perhaps regulated by, the major histocompatibility complex[2,3]. We now present the tissue typing results from a group of patients with testicular cancer. The results show an abnormally high antigen frequency for HLA-Dw7 in patients with testicular teratocarcinoma.

The study population consisted of 61 patients from the University of Minnesota. Thirty patients had seminoma, 27 had embryonal cell carcinoma, 26 had teratocarcinoma, and 7 patients had choriocarcinoma (some of the patients had mixed tumours). All patients had completed treatment (surgery, radiotherapy, and/or chemotherapy) and were clinically free of disease at the time of testing. Peripheral blood lymphocytes were obtained on each individual by Ficoll–Hypaque density centrifugation[4] and frozen at a controlled rate in 10% dimethyl sulphoxide and RPMI 1640 with 25 mM HEPES (Microbiological Associates) supplemented with 20% pooled human serum (PHS), heparin (20 IU ml^{-1}), L-glutamine (0.2 mg ml^{-1}), streptomycin (100 μg ml^{-1}) and penicillin (100 IU ml^{-1}). The cells were stored in vapour phase liquid nitrogen until ready for analysis at which time they were thawed rapidly at 37 °C and diluted with 5% PHS, washed twice, and resuspended in culture media for testing. All cells were tested for 52 HLA-A, HLA-B and HLA-C specificities by the NIH standard technique[5]. HLA-D assignments were made by homozygous typing using at least 22 typing cells covering all HLA-D specificities except HLA-Dw8 and HLA-Dw9. Homozygous typing was performed in round-bottomed microtitre plates using 5×10^4 responders and 5×10^4 stimulator typing cells inactivated with 5,000 rad from a ^{137}Cs source (3.2 min). Cells were suspended in 0.2 ml of 20% PHS and incubated in a 37 °C, 5% CO_2 and air atmosphere for 6 days. They were then terminated immediately after a 6-h pulse with 1.0 μCi ^3H-TdR (6.7 Ci $mmol^{-1}$, NEN). Cells were collected on an automated harvester. Results were 'double-normalised' and typing responses were assigned to those cells that gave less than 35% DNV. The reactivity of patient cells was checked by stimulation with purified PHA (HA-17) (Wellcome), and only those patients responding in the same range as normal control cells (typed simultaneously with the tumour patients) were included for the HLA-D typing results. Antigen frequencies were then calculated for the tumour group as a whole as well as for each of the groups of patients with seminoma, embryonal cell carcinoma and teratocarcinoma, and compared to normal control values ($n = 561$ for HLA-A and HLA-B, 171 for HLA-C and 150 for HLA-D specificities) obtained from a random population of the same geographical area as the tumour patients. Significance was determined by χ^2 analysis and P values were corrected to account for chance significance; $P < 0.01$ was considered significant[6].

The antigen frequency of HLA-Dw7 was significantly increased in the teratocarcinoma group (12/26, $P < 0.01$) as compared to normal control values (14/150) (Table 1); the difference, however, was not detected in either of the seminoma (7/24, not significant), choriocarcinoma (0/7, NS) or embryonal cell carcinoma groups (7/24, NS) or the total patient group (15/53, NS). (Six patients were eliminated from the original seminoma group (30) and 3 patients from the original embryonal carcinoma group (27) for HLA-D typing because of inadequate reactivity to HA-17.) The relative risk (which is a calculated term that represents the risk of developing a disease in an individual possessing a particular HLA antigen as

Table 1 Frequency of HLA-D determinants in patients with testicular teratocarcinoma and controls

Determinant	Normal controls (%)	Patients with teratocarcinoma (%)	*P* value
Dw1	19/150 (13)	7/26 (27)	NS
Dw2	19/150 (13)	3/26 (12)	NS
Dw3	21/150 (14)	6/26 (23)	NS
Dw4	12/150 (8)	2/26 (8)	NS
Dw5	7/100 (7)	1/26 (4)	NS
Dw6	0/50 (0)	1/26 (4)	NS
Dw7	14/150 (9)	12/26 (46)	<0.01
Dw10	5/150 (3)	0/26 (0)	NS
Dw11	18/150 (12)	0/26 (0)	NS
Single blank	69/168 (41)	8/26 (31)	NS
Double blank	30/168 (18)	6/26 (23)	NS

HLA-D typing was performed as described in the text. Lymphocyte responsiveness for each patient was checked by response to purified PHA (HA-17). 5×10^4 cells were incubated in 0.2 ml of 1:100 HA-17. Cultures were terminated at 96 h immediately after a 6-h pulse with 1.0 μCi ^{3}H-TdR (6.7 Ci mmol^{-1}) Only those cells responding in the range of normal controls were included in the HLA-D typing results. All HLA-Dw7 assignments were made by typing responses to HTC JB-12 (submitted to the Seventh International Histocompatibility Workshop, Oxford, as 7w542) and HTC 56 (submitted as 7w534). One teratocarcinoma patient was assigned HLA-Dw1, Dw6, Dw7 and included in the HLA-Dw7 group; however, if this patient were excluded from analysis, the increased HLA-Dw7 antigen frequency would still be significant (11/25, $P<0.01$). NS, not significant

compared to an individual lacking that antigen) between HLA-Dw7 and teratocarcinoma was 8.32. The association between HLA-Dw7 and teratocarcinoma, although statistically significant, should be interpreted with the understanding that the HLA-D assignments are based on reactivity of responder (tumour patients') T cells which can be affected by the presence of cancer as well as its various modes of treatment. In order to reduce this source of error, lymphocyte reactivity from each study patient was checked with purified PHA (HA-17) and only those responding in the same range as normal control cells were included in the HLA-D typing results.

The biological significance of this as well as other disease associations with HLA is, as yet, unknown. However, four possible mechanisms have been suggested. (1) HLA antigens may cross-react with pathogen antigens leading to cross-tolerance; the pathogen, then would not be subjected to the host's immune mechanisms. (2) HLA antigens may function as cell surface receptors for particular pathogens similar to the association between malaria susceptibility and Duffy red blood cell antigens[7]. (3) There may be a genetic linkage between genes controlling the expression of HLA antigens (which serve as markers) and genes controlling immune responsiveness of the host and likewise, (4) HLA antigens may serve as markers for specific genes causing deficiency of specific normal proteins similar to the HLA linked genes regulating levels of second and fourth components of complement[8].

Irrespective of which of these mechanisms apply, the association between HLA and testicular teratocarcinoma is especially interesting for two reasons—first, it has been suggested that heredity may be an aetiologic factor in the development of testicular cancer because at least 18 cases of familial testicular cancer have been reported[9]. And second, there is a relationship between teratocarcinoma and H-2 in the mouse[10]; specifically, the H-2 linked T/t complex which exerts control over embryonic differentiation, male reproduction including sterility and transmission ratio distortion, and crossing over frequency between T/t and H-2 (refs 11, 12) also controls the expression of an embryonic antigen, F9, that is expressed on adult teratocarcinoma cells[13,14]. In addition, the F9 antigen is similar to H-2 antigen in molecular weight and subunit structure and may, in fact, represent an embryonic equivalent of histocompatibility antigens[15].

The association between the human major histocompatibility complex (HLA) and testicular teratocarcinoma is therefore of interest considering the homology between mouse and the human major histocompatibility complex. First, because F9 and H-2 K and D region antigens have closely associated molecular and subunit structures[15], a similar teratocarcinoma associated antigen in the human may cross-react with HLA (HLA-Dw7) leading to a state of 'cross-tolerance' which would support one of the theories of HLA and disease association. Second, considering the established relationship between the major histocompatibility complex, the T/t complex, and teratocarcinoma in the mouse system, a relationship between HLA-Dw7 and teratocarcinoma may add support to the existence of a MHC linked 'T/t like' region in the human. This concept is further supported by the association between HLA and spina bifida which may be considered to have morphological similarities to those controlled by the T/t complex. In addition, there are antigens cross reacting with F9 on human sperm[17] as well as on human teratocarcinoma[18].

Thus, in the mouse there is a structural and developmental association between a teratocarcinoma associated antigen (F9) and the major histocompatibility complex. The association of HLA-Dw7 with testicular teratocarcinoma could lead to similar studies in the human that will further explain the function of the human major histocompatability complex.

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Specific antibody-secreting cells generated from cells producing immunoglobulins without detected antibody function

STUDIES of immune responses in rabbits, rats and mice have led us to describe a population of differentiated immunocytes synthesising and secreting immunoglobulins without detectable antibody function for the antigens injected[1] (IFC). These cells were antigen induced and appeared before the antibody-forming cells (AFC). The proliferation of IFC after antigenic stimulation can be explained by several hypotheses, the most plausible being that antigen-stimulated T cells release nonspecific factors which trigger clones of B cells bearing membrane receptors unrelated to the inoculated antigen. Such an explanation has been advanced by several authors[2,3] but experiments performed in our laboratory do not support such polyclonal activation. Indeed, some cells contained both antibodies and immunoglobulins without detectable antibody function in different areas of their cytoplasm[1,4]. Furthermore, some common idiotypic specificities were expressed by both AFC and IFC (ref. 5). Based on these findings, and additional studies on the developmental kinetics of IFC and AFC in animals with different immunological status, which indicate that there is a close relationship between these two cell populations, we put forward the hypothesis that AFC might be recruited among IFC. That is, that these two cell types represent two stages of differentiation of the same cell population. To test this assumption directly we performed experiments at the single cell level with micromanipulated and individually cultured IFC and found that at least some of these cells generate specific AFC.

Adult C57BL/6 strain mice were immunised by injection of horseradish peroxidase (PO) emulsified in Freund's complete adjuvant into both hind footpads. Six days later, cell suspensions were prepared from their popliteal lymph nodes and tested for the presence of AFC and IFC. Anti-peroxidase antibody-secreting cells (AFC) and immunoglobulin-secreting cells were detected by Cunningham's[6] and reverse[7,8] local haemolysis plaque assays respectively (see legend to Table 1). At this time, 801 ± 301 IFC per 10^6 viable lymph node cells were present, but no AFC were found. The lymphoid cells were mass cultured for 24 h to select the most resistant cells under *in vitro* conditions. After this culture, the number of IFC did not vary and very few anti-PO and anti-sheep red blood cell (SRBC) AFC appeared (Table 1). Some of these IFC were micromanipulated, transferred into separate wells of polyacrylamide rafts[14,15] and individually cultured in the conditions described in Table 2, in the presence of irradiated syngeneic splenocytes or thymocytes as feeder layer and horse red blood cells covalently linked with PO (HRBC–PO) as stimulating antigen. The scarcity of indirect anti-PO and anti-SRBC AFC detected at the time of micromanipulation made it highly improbable that such PFC would be micromanipulated (Table 1). Almost all the micromanipulated cells can therefore be considered as cells really secreting immunoglobulins without detectable antibody function.

Table 1 Number of IFC, anti-peroxidase and anti-SRBC AFC per 10^6 viable lymph node cells after 24 h of mass culture*

IFC	Anti-peroxidase AFC Direct plaques	Anti-peroxidase AFC Indirect plaques	Anti-SRBC AFC Direct plaques	Anti-SRBC AFC Indirect plaques
872 ± 474	1 ± 4	4 ± 6	1 ± 2	1 ± 3

C57B1/6 mice were injected in both hind footpads with 250 μg of horseradish peroxidase (PO) emulsified in Freund's complete adjuvant and killed 6 d later. Popliteal lymph nodes were removed, teased and cells were mass cultured in conditions reported in detail previously[9–11]. Briefly, 8×10^6 viable lymphoid cells were cultured without shaking in 35 mm Petri dishes containing 2 ml Click's medium[12] supplemented with LPS (*Escherichia coli* 055B5 lipopolysaccharide, Difco) at a final concentration of 4 μg ml^{-1} and with PO covalently linked to horse red blood cells[13] (HRBC–PO, 5×10^6 per dish). The cells were collected 24 h later and the anti-PO, anti-sheep red blood cell (SRBC) AFC and immunoglobulin-secreting cells were detected by local haemolysis plaque assay, the indicators used being either SRBC covalently coupled to PO (ref. 13), SRBC or SRBC coated with rabbit hybrid antibody $(\text{Fab}')_2$ fragments with dual specificity to SRBC and mouse immunoglobulins (refs 7, 8). SRBC adsorbed guinea pig serum was used as source of complement. To reveal the indirect AFC and immunoglobulin-secreting cells, a rabbit anti-mouse Ig antiserum was added to the mixture (final dilution used: 1/400).

* Arithmetic means of 19–24 different experiments ± one standard deviation. In each experiment the lymph nodes of 5–12 mice were pooled.

Table 2 Percentage of IFC which generate anti-PO AFC or which change into anti-PO AFC

No. of micromanipulated IFC	Presence(+) or absence(−) of peroxidase inhibitor	No. and percentage (in parentheses) of wells containing AFC after 48 h of microculture	Total no. of haemolytic plaques detected with SRBC–PO after 48 h of microculture
392	−	90 (23)	345
119	+	2 (1.7)	3

Lymphoid cells were collected after 24 h of mass culture (see legend to Table 1). Revealed IFC were micromanipulated from the centre of haemolytic plaques by a previously described procedure[10,11] and transferred into separate wells of polyacrylamide plates previously seeded with 10^3 to 10^4 irradiated (1,500 rad) normal C57BL/6 mouse splenocytes or thymocytes as feeder layer and 10^3 to 5×10^3 HRBC–PO as antigen. The medium used for the microculture was composed of 50% fresh Click's medium and 50% previously filtered supernatant of the mass culture. After 48 h of microculture, the content of each well was removed and mixed with SRBC–PO and complement. Cells lysing these red cells were detected by Cunningham's haemolysis plaque assay. The specificity of the molecules secreted by the progeny of microcultured cells capable of lysing SRBC–PO was tested in control experiments by adding soluble peroxidase as inhibitor to the revealing mixture (final concentration 70 μg ml^{-1}).

After 48 h of culture, 90 out of 392 micromanipulated IFC (23%) were transformed (in 34% of positive wells only one haemolytic plaque was detected) into cells able to lyse SRBC–PO or generated daughter PFC lysing these erythrocytes. Only some of the individually cultured cells yielded a PFC progeny. In

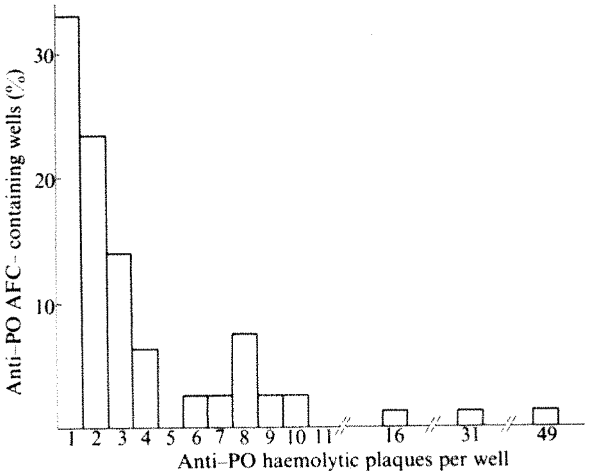

Fig. 1 Distribution of the number of anti-PO haemolytic plaques found in each positive well seeded 48 h before with a single micromanipulated IFC.

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previous experiments of this type, about 10–20% of parental PFC generated daughter PFC (refs 11, 15) the others could be either terminal cells, cells generating memory cells or cells injured by micromanipulation procedure. It has been shown[11] that PFC are derived from micromanipulated cells and not from the irradiated cells used as the feeder layer, and this was further tested in the present study (unpublished results). Thus, no anti-PO AFC was found in wells containing only irradiated splenocytes or thymocytes and HRBC–PO, irradiated splenocytes or thymocytes used as the feeder layer were equally effective for the generation of AFC from IFC, and if the number of irradiated splenocytes was varied from 10^3 to 10^4 cells per well, the same percentage of wells containing anti-PO AFC was found.

Of the wells containing anti-PO AFC, 66% had more than one haemolytic plaque (see Fig. 1). Often, the centre of large haemolytic plaques contained several clustered cells. It is therefore likely that one or more divisions of the micromanipulated cells were needed before the secretion of anti-PO antibodies by the daughter cells could be detected.

Table 3 Some AFC derive from IFC. This event requires the presence of the immunising antigen

No. of micromanipulated IFC	Presence or absence of PO in the microculture	No. of wells containing AFC after 48 h of microculture	Total no. of haemolytic plaques detected on SRBC–PO after 48 h of microculture
110	HRBC–PO	30 (27.3%)	174
127	HRBC	2 (1.6%)	8

Micromanipulated IFC were transferred into separate wells of polyacrylamide rafts containing 5×10^3 irradiated (1,500 rad) normal C57BL/6 mouse splenocytes and either 2.5×10^3 HRBC–PO, or 2.5×10^3 native HRBC. For conditions of the microculture and detection of daughter PFC see Table 2.

The specificity of the antibody secreted by the daughter cells lysing the SRBC–PO was checked by adding soluble peroxidase in the revealing mixture. In these control experiments, only 2 out of 119 wells tested contained 3 haemolytic plaques, (compared to 90 out of 392) (Table 2). The 97% inhibition of haemolytic plaques when soluble peroxidase was present in the revealing mixture and the absence of antigenic determinants shared by SRBC and PO (ref. 8 and unpublished results) demonstrate that cells lysing the SRBC–PO were true anti-PO antibody producers.

To determine whether the presence of the immunising antigen was critical to the generation of AFC from IFC, some of the latter cells were micromanipulated and transferred to wells containing irradiated cells and native HRBC (Table 3). Control experiments where IFC were microcultured into wells containing HRBC–PO, were run simultaneously. Only the IFC cultured in the presence of HRBC–PO generated a large number of anti-PO AFC. In contrast, very few anti-PO haemolytic plaques were detected in wells seeded with native HRBC (25 times less).

The experiments reported in the present paper clearly demonstrate that some IFC taken from mice immunised with PO generate anti-PO AFC or change into anti-PO AFC. This event seems to be associated with one or several cell divisions and requires the presence of the antigen. It appears, therefore, that IFC and AFC do represent different stages of differentiation of the same cell population. One explanation is that IFC and precursors of IFC are monopotent cells, that is, there is only one antibody-forming potentiality per cell, the IFC synthesising and secreting non-functional precursors of antibodies. Such an explanation is not substantiated by the biochemical study we have performed on these cells[16]. Indeed it has not been possible to detect any particular composition or molecular weight for the immunoglobulins synthesised by the IFC. Furthermore the finding that the presence of the antigen is required for this step of differentiation to occur makes this type of explanation improbable. Alternatively IFC and precursors of IFC may be multipotent cells, that is, they synthesise and secrete immunoglobulins with multiple specificities. During differentiation, and under the selective influence of the antigen, they acquire a specialisation, that is, they synthesise monospecific antibody molecules. The finding that the presence of the antigen is required for this step of differentiation to occur lends further weight to such a view. This event may require genetic rearrangement, occurring during cell division.

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Murine embryonic B lymphocyte development in the placenta

B LYMPHOCYTES derive from pluripotent stem cells which give rise to erythrocytes, granulocytes, monocytes, macrophages and T and B lymphocytes[1,2]. Stem cells have been found in yolk sac, fetal liver, bone marrow, spleen and blood. During ontogeny from stem cells to mature B cells, precursor B cells (pre-B cells) can be found. Various types of pre-B cell can be identified. There are those which already produce and release immunoglobulin (Ig), but which are morphologically different from mature B cells[3,4]. Using surface-bound Ig as a marker for the development of mature B cells, it has previously been found that fetal liver, but not yolk sac, could develop mature B cells *in vitro*[5,6]. A second type of pre-B cell can be characterised functionally as not being reactive to antigen or mitogens but which, on adoptive transfer into irradiated recipients or following *in vitro* culture, develops into a reactive cell[7–9]. When stimulated by antigens or mitogens, such mature, reactive surface Ig-bearing resting B lymphocytes develop into IgM-, IgG-

Table 1 H-2 haplotype of LPS-induced PFC*

Placenta (embryo)	Treatment	Complement only	Anti-DBA/2J (H-2^b anti-H-2^d)	Anti-C57BL/6J (H-2^d anti-H-2^b)
(C57BL/6J × DBA/2J)F$_1$		500 ± 100	60 ± 30	50 ± 40
(H-2^b × H-2^d)	(Reduction)		(88%)	(90%)
Spleen (adult, mother)				
C57BL/6J		850 ± 150	820 ± 200	45 ± 20
(H-2^b)	(Reduction)		(4%)	(95%)
Spleen (adult, father)				
DBA/2J		550 ± 100	20 ± 15	480 ± 100
(H-2^d)	(Reduction)		(97%)	(12%)
Spleen (adult, F1)				
(C57BL/6J × DBA/2J)F$_1$		920 ± 120	90 ± 30	40 ± 20
(H-2^b × H-2^d)	(Reduction)		(90%)	(95%)

* Duplicate aliquots of the IgM-secreting PFC at the peak of the response were first incubated with C57BL/6J (anti-DBA/2J spleen cell) serum (H-2^b anti-H-2^d) or with DBA/2J (anti-C57BL/6J spleen cell) serum (H-2^d anti-H-2^b) (both gifts from Dr H. von Boehmer) for 30 min on ice, thereafter washed and then incubated with appropriately diluted rabbit complement for 30 min at 37 °C. Finally, the number of IgM-secreting PFC was determined in the protein A-SRC plaque assay[11]. PFC were treated this way for placenta cell cultures (at day 12 of gestation) at time equivalent to the time of birth (day 19 of gestation) in culture, and at day 5 of culture for spleen cells grown from 3×10^5 cells ml^{-1} in the presence of LPS.

and IgA-secreting plasma cells, which can be detected using a haemolytic plaque assay as plaque-forming cells (PFC)[10,11]. Pre-B cells which develop into antigen- or mitogen-reactive cells have been localised in fetal liver, bone marrow and spleen. The earliest that pre-B cells had previously been identified during embryogenesis of the mouse was at about day 12 or 13, but I report here the detection of IgM- and IgG-secreting PFC in mitogen-stimulated cultures of single cell suspensions of mouse placenta 9–14 d old. Placenta is thus identified as a new site of B-lymphocyte development which, during embryogenesis, precedes B-cell generation in fetal liver.

The culture technique used facilitates *in vitro* development of pre-B cells from the placenta to mature B-cells which can subsequently be stimulated by the B-cell mitogen lipopolysaccharide (LPS) to IgM- and IgG-secreting cells. Placentae were obtained from timed pregnant C57BL/6J female (H-2^b) mice, mated with DBA/2J male (H-2^d) mice. The embryo was first removed from the uterine wall and then the ectoplacental cone was dissected away from the uterus, and thereafter, from the yolk sac and embryo. They were rinsed extensively with balanced salt solution (BSS) and then dispersed into single cell suspensions by cutting the cones into small pieces with scissors, followed by passaging of the placental pieces through a stainless screen (200 mesh) with the aid of the piston of a 5-ml plastic syringe. After washing three times with cold BSS, the placental cells were suspended at 5×10^6 cells ml^{-1} in medium containing LPS. Culture conditions were those described previously for fetal liver cells[8], except that dextran sulphate was added. The addition of dextran sulphate increased the total LPS-induced IgM-PFC response of placental cells five- to tenfold (details to be published elsewhere), but the development of IgM- and IgG-PFC in cultures of placental cells was still dependent on the presence of the B-cell mitogen LPS.

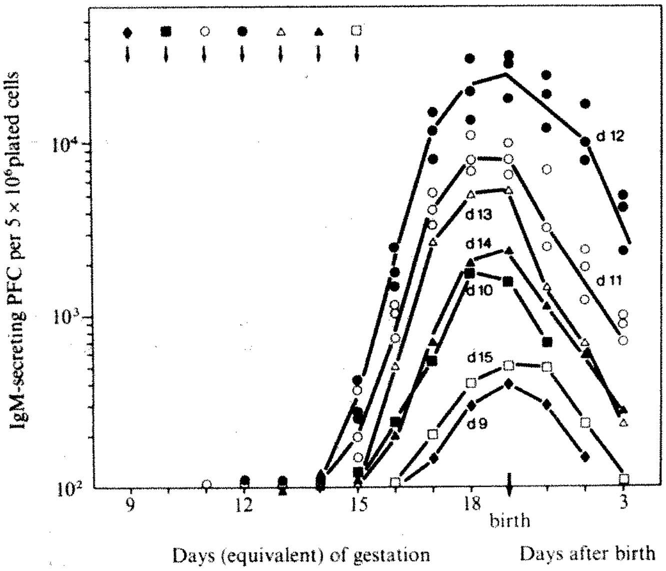

Fig. 1 The development of IgM-secreting PFC (determined with the protein A-SRC assay[11]) in suspension cultures of placenta at different stages of gestation (◆, day 9; ■, day 10; ○, day 11 (3 experiments); ●, day 12 (3 experiments); △, day 13; ▲, day 14) in the presence of dextran sulphate (100 μg ml^{-1}; MW 500,000; Serva) and LPS (50 μg ml^{-1}; a gift from Drs C. Galanos and O. Lüderitz, Max-Planck Institut fur Immunbiologie, Freiburg, West Germany) assayed at various days during culture.

Cells obtained from placenta at any time during gestation (between days 9 and 14) yielded a peak of IgM-secreting PFC in culture at the time corresponding to day 19 of gestation (Fig. 1). This was followed by the peak of IgG-secreting PFC 2–5 d later (not shown). As shown previously[8], the largest number of IgM-secreting PFC developing from mature B-cells is usually observed in such cultures 4–5 d after mitogenic stimulation. If it is assumed that maturation of B cells from placenta *in vitro* parallels in time their maturation *in vivo*, then placental cells, kept either *in vivo* or *in vitro*, acquire LPS reactivity probably at the equivalent of days 14–15 of gestation. The highest number of precursor B cells was observed in placental cells removed on day 12 of gestation, as estimated by the total number of PFC obtained in the mitogenic response (Fig. 1).

The majority of all IgM-secreting PFC developing in placental cell cultures expressed the embryonic H-2^b and H-2^d haplotypes (Table 1) following LPS stimulation. This excludes the possibility that the LPS-reactive cells were contaminating maternal (H-2^b) cells.

The frequency with which precursor cells in the placenta developed LPS reactivity and then into IgM-secreting PFC in culture, was determined by limiting dilution analyses in the presence of rat thymus 'filler' cells (3×10^6 per ml) (ref. 9). Placental cells at day 10 of gestation contained one precursor in 2×10^4 cells; at day 11, one in 4×10^3; at day 12, one in 8×10^2; at day 13, one in 5×10^3; and at day 14, one in 10^5 cells. Placenta, therefore, precedes fetal liver as an organ of embryonic B-cell development by several days[9]. As placental B-cell development declines, it rises in fetal liver (Fig. 2).

B-lymphocyte development in the mammalian embryo has been recognised to be multifocal. Furthermore, it is now evident that the sites of B-lymphocyte development change with the stage of embryogenesis. A first wave of B-cell differentiation in the placenta up to day 12 of gestation is followed by a second wave in fetal liver, which continues to birth. Thereafter, spleen and bone marrow take over as major sites of B-cell development. Placental B-cell generation begins concomitantly with the development of blood islands in yolk sac and ceases (at day 12) when haematopoiesis ceases in the yolk sac. Placenta and yolk sac are in intimate association during this time of embryogenesis[12]. It is, therefore, possible that pluripotent stem cells or B-cell precursors migrate from yolk sac to placenta for further differentiation along the B-cell pathway.

Yolk sac is known to contain pluripotent stem cells from which mature erythrocytes, granulocytes, monocytes, megakaryocytes and lymphocytes arise by differentiation. Cell-marker experiments have shown that stem cells migrate, and are thereby seeded into embryonic myeloid organs and primary lymphoid organs, such as fetal liver, bone marrow, spleen and thymus[13]. Stem cells themselves are thought to be uncommitted; it has been suggested that their differentiation into the various types of blood cells depends on the inductive influence of the organ rudiment into which they migrate[14]. In avian blood cell differentiation, it is possible that the epithelium of the bursa of Fabricius could have such an inductive effect, although there is no indication as to the genetic loci which may control the expression of the inducing signals on such epithelial cells. Neither is anything known of the possible genetic control of murine B-cell development through genes controlling the expression of B-cell differentiation-inducing signals. If such differentiation-inducing structures exist, they are probably expressed on mesenchymal-endodermal elements[15] of placenta, fetal liver, bone marrow and spleen. B-cell differentiation in the placenta, however, may offer embryonic stem cells the possibility to differentiate in response to signals which are encoded in the maternal genes, expressed on maternal endoderm within the placenta.

The site of B-cell differentiation certainly does not depend on the site of stem-cell generation. Stem cells could migrate to the placenta either from yolk sac[13] or from another embryonic site[16], particularly since the embryo, at day 9 of gestation, has developed a heart and a circulatory system in which cells can migrate from yolk sac to embryo and to placenta and back.

The ability of placental cells from as early as day 9 of gestation to differentiate into mature B-cells *in vitro* offers exciting new experimental possibilities of probing the repertoire of variable regions of heavy and light chains, expressed very early in embryonic development, which may be close to or identical to the repertoire carried in the germ line. If cellular contact between stem cells and 'environment' is needed for B-cell differentiation, then the rudiment of placenta may be one of the earliest sites on which B-cell differentiation occurs.

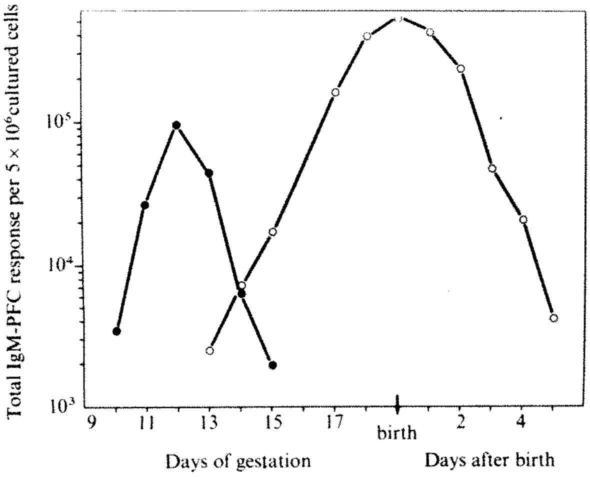

Fig. 2 LPS responsiveness of placentae (●) and fetal liver (○) (see ref. 9) cells taken in culture in the presence of LPS at different stages of development. IgM-secreting PFC responses were determined each day from the equivalent of day 15 of gestation until the equivalent of day 3 after birth of culture (see also Fig. 1). The sum of PFC determined at the different days is given as total PFC response.

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Augmentation by interferon of human natural and antibody-dependent cell-mediated cytotoxicity

LYMPHOCYTES from normal individuals may have considerable levels of cytotoxic reactivity against tumour cells, mediated by NK (natural killer) cells[1]. There are many similarities between NK cells and the effector cells (K cells) mediating antibody-dependent cell-mediated cytotoxicity (ADCC) against tumour target cells[2,3], and they may in fact be identical cells. *In vivo* inoculation of rodents with viruses[4,5], immunostimulants[4,6–8], tumour cells[4] and other interferon inducers[8] results in augmented NK activity. Interferon (IF) was shown to have a major role in rapidly boosting mouse NK activity, both *in vivo*[9,10] and *in vitro*[9]. Trinchieri and Santoli[11] have suggested that IF could also augment human NK activity. However, the preparations used might have contained a variety of other lymphokines. The present study shows that IF can indeed augment human NK activity and also ADCC.

Peripheral blood mononuclear leukocytes (PBL) were collected from normal adult donors by centrifugation of heparinised blood on Ficoll–Hypaque gradients. Cytotoxicity was measured in a 4-h ^{51}Cr-release assay, with K562 used as the target cell for NK, and Chang liver cells coated with rabbit IgG antibody as the target for ADCC[2]. All experiments were carried out with several effector : target cell ratios, and activity was expressed in lytic units (LU) per 10^7 cells, 1 LU being defined as the number of cells required to produce 30% specific cytotoxicity[2]. Three different, partially purified, human IF preparations were used: lymphoblastoid IF (IF_{LB}), induced by Sendai virus (batch LNI/77/3A; 1.57×10^6 U IF per mg protein); leukocyte IF (IF_{LE}), induced in human leukocytes by Sendai virus[12] (lot 1, 1.1×10^6 U per mg protein); and fibroblast IF (IF_F), induced by poly I : C (3×10^5 U IF per mg protein).

Two rabbit antibodies against human IF were used: anti IF_F (pool 29–33, anti-IF titre of 10^3 per ml) and anti-IF_{LE} (ref. 13) (R24, titre of 10^4 per ml). To evaluate the effects of the IF preparations, PBL ($2–5 \times 10^6$ per ml) were preincubated at 37 °C with IF (usually 10^3–10^4 U ml^{-1}) and then washed before

Table 1 Effect of various human interferon preparations on cytotoxic activity of peripheral blood mononuclear cells

		NK activity			ADCC activity		
Donor	Treatment	% Cytotoxicity* 7:1	Lytic units per 10^7 cells	% Of control lytic units	% Cytotoxicity† 7:1	Lytic units per 10^7 cells	% Of control lytic units
1	Control	17.7	123	—	20.8	196	—
	IF_F (10^4 U)	35.8	355	289	31.5	207	106
	IF_{LB} (10^4 U)	37.2	405	329	29.2	179	91
	IF_{LE} (10^4 U)	33.0	237	192	34.9	277	141
		16.7:1			16.7:1		
2	Control	13.3	16	—	16.9	16	—
	IF_F (10^3 U)	26.6	64	400	20.4	39.4	250
	IF_F (10 U)	17.9	25.4	159	16.0	19	119
	IF_{LB} (10^3 U)	21.3	33.3	208	35.8	104	656
	IF_{LB} (10 U)	20.9	32.5	203	25.7	76.6	480
	IF_{LE} (10^3 U)	28.8	63	388	22.2	35.5	222
	IF_{LE} (10 U)	18.8	43	269	29.4	40	225

Interferon preparations: IF_F, interferon prepared from fibroblasts, IF_{LB}, interferon prepared from a lymphoblastoid cell line; IF_{LE}, interferon prepared from leukocytes. Preparations from donor 1 were treated for 1 h, those from donor 2 for 18 h.

* Baseline relase of ^{51}Cr from K562 target cells was 8.9%. Percentages given were obtained at one of the several effector : target cell ratios tested.

† Baseline release of ^{51}Cr from antibody-coated Chang target cells was 10.4%. Percentages given were obtained at one of the several effector : target cell ratios tested.

Table 2 Summary of *in vitro* effects of interferon on NK- and K-cell activities of peripheral blood lymphocytes of normal donors

	Natural cytotoxicity			ADCC		
Treatment time (h)	Median	Range	No. expt >150% of control/total expt	Median	Range	No. expt >150% of control/total expt
1	160*	60–1626	11/22	141	91–455	3/12
18–20	168	75–292	7/11	164	90–494	8/11

* Activity after treatment as per cent of activity (calculated as lytic units per 10^7 cells) in untreated control lymphocytes.

Table 3 Effect of anti-interferon on augmentation of cytotoxic activity by IF_{LB}

		NK activity		ADCC activity	
	Treatment	Lytic units per 10^7 cells	% Of control	Lytic units per 10^7 cells	% Of control
Expt 1	Control	13.3	—	44.4	—
	IF_{LB} (1,000 U)	40.9	297	70.5	159
	IF_{LB}+anti-IF_F (1:10)	2.1	5	0.4	<1
	IF_{LB}+anti-IF_F (1:40)	10.6	74	16.2	23
	IF_{LB}+anti-IF_{LB} (1:10)	0.0	<1	0.0	<1
	IF_{LB}+anti-IF_{LB} (1:40)	1.2	3	1.5	2
	Anti-IF_F (1:10)	0.1	<1	0.0	<1
	Anti-IF_{LB} (1:10)	0.2	1	0.0	<1
Expt 2	Control	70.2	—	36.7	—
	IF_{LB} (1,000 U)	170.0	242	51.2	148
	IF_{LB}+anti-IF_F (1:100)	86.1	122	31.6	86
	IF_{LB}+anti-IF_{LB} (1:100)	51.6	73	6.9	19
	Anti-IF_F (1:100)	69.3	99	14.9	41
	Anti-IF_{LB} (1:100)	39.5	56	1.1	3

Expt 1, preincubation of PBL at 37 °C for 18 h; expt 2, preincubation of PBL from another donor at 37 °C for 1 h.

testing for cytotoxicity. To evaluate the effects of anti-IF, various dilutions of antisera were added to the preincubation mixtures.

With some donors, augmentation in NK by IF was seen within a few minutes, with a peak at about 1 h (for example donor 2, Table 1). The three IF preparations gave parallel results, each showing effects down to 10 U ml^{-1} or less. Table 2 summarises our overall results with several normal donors. NK activity of some donors was boosted strongly, as much as 16-fold, whereas that of others was unaffected. The NK activity of a similar proportion of donors was appreciably (>150% of control LU per 10^7 cells) boosted at 1 h and 18–20 h. In contrast, boosting of K-cell activity occurred more frequently after overnight incubation. The variations in responsiveness of different donors may be analogous to experiments in mice[9], in which sensitivity to IF varied among strains and was influenced by previous *in vivo* exposure to activating stimuli. Our data on NK activity agree fairly well with those of Trinchieri and Santoli[11], but it is not clear why they failed to detect the boosting of K-cell activity, as they preincubated their cells for 18 h.

To obtain further support for the role of IF as the active molecule in augmentation of human NK- and K-cell activities, we tested whether antibodies to IF could block the effects of IF. IF_{LB} was used for these experiments, so as to have an IF preparation from a source different from those used for immunisations, making it less likely that antibodies to other contaminating proteins would be responsible for the effects. Both antisera were able to block the boosting by IF_{LB} of NK- and K-cell activities completely (Table 3). In fact, these reagents caused a decrease to below unboosted levels. This was not due to toxicity, as after removal of the reagents, the cells could still be stimulated by IF and were normally responsive to the mitogenic effects of phytohaemagglutinin. Inhibition by IF–anti-IF complexes seemed unlikely as we have never been able to detect more than 5 U of IF per ml medium after 18 h incubation of PBL. However, to control further for this possibility, we compared the effects of anti-interferon with those of anti-bovine serum albumin (BSA), which would be expected to form complexes with the albumin in the fetal bovine serum (Table 4).

Table 4 Comparison of effects of anti-interferon and anti-bovine serum albumin on augmentation of cytotoxic activity by lymphoblastoid interferon

	NK activity		ADCC activity	
Treatment (18 h at 37 °C)	Lytic units per 10^7 cells	% Of control	Lytic units per 10^7 cells	% Of control
Control	111.1	—	175	—
IF_{LB} (1,000 U)	191.6*	172	230.9*	143
IF_{LB} + anti-IF_F (1:100)	95.5	86	25.7*	15
Anti-IF_F (1:100)	28.8*	26	5.0*	3
IF_{LB} + anti-BSA (1:100)	187.1*	169	123*	70
Anti-BSA (1:100)	99.7	89	38.4*	22

* Significantly different from untreated control, $P < 0.05$

In contrast to the inhibition of NK activity and blocking by boosting by IF, the anti-BSA has no significant effects, although it did inhibit ADCC and its boosting by IF, presumably due to the known inhibitory effects of immune complexes on ADCC.

Because of the effect of complexes on ADCC and our inability to prepare $F(ab')_2$ fragments of anti-interferon (sufficient quantities of the reagents were not available), the effects of anti-interferon on boosting of ADCC by interferon cannot be readily interpreted. However, the marked effects of anti-interferon on NK activity seem to be specific and may indicate a normal role of IF in maintaining this effector cell function. As brief preincubation of IF with lymphocytes can induce activation of NK cells, it seems likely that surface-bound IF can lead to activation during the assay. The results with anti-IF suggest that some spontaneous NK activity results from activation during culture by IF bound *in vivo* or secreted during the assay.

The IF-activated effector cells had the same characteristics as NK cells. After incubation with IF for 18 h and before testing, monocytes were removed by passage of the cells over Sephadex G-10. No removal of cytotoxic activity against K562 or against antibody-coated Chang cells was observed after such treatment. Like NK and K cells[2], the IF-induced cytotoxic cells had Fc receptors for IgG, with complete removal of reactivity by adsorption on immune-complex monolayers.

The rapid and strong potentiation of cytotoxic effector cell activities by IF has important implications for *in vivo* resistance against tumour growth. These effects need to be seriously considered as possible mechanisms for the anti-tumour effects of a variety of immune adjuvants—as well as those of IF itself—which are being used in immunotherapy trials.

Human IF preparations were obtained from the NCI Interferon Working Group, and rabbit anti-IF antibodies from the Antiviral Substances Program, NIAID.

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Primary sensitisation and restimulation of human lymphocytes with soluble antigen *in vitro*

RESEARCH with human lymphocytes is restricted by ethical constraints on sensitising individuals *in vivo* before *in vitro* experiments. In humans specific restimulation of proliferative responses was first shown using allogeneic lymphocytes as antigen and has since been developed into the primed lymphocyte test[1]. In a model similar to this test, again using cell-bound antigens, Newman *et al.*[2] showed sensitisation and restimulation by hapten-modified autologous cells. With soluble protein antigens and lymphocytes from donors who had been sensitised to these antigens *in vivo*, Levis and Dattner[3] showed specific restimulation of proliferative responses, after the cells had been cultured in the presence of antigen for 10 or more days. We report here that, using a similar culture method, but cells from individuals who have not been sensitised *in vivo* to the relevant protein antigens, we have been able to obtain *in vitro* sensitisation and specific restimulation, sensitisation of the lymphocytes being determined by a subsequent specific proliferative response.

Lymphocytes were isolated by standard techniques from human peripheral blood and cultured for 10 d with the sensitising antigen. Cells were then washed and restimulated by culturing them for a further 2 or 3 d in the presence of the same or different antigens. Proliferative responses were measured by the incorporation of tritiated thymidine. Antigens used were keyhole limpet haemocyanin (KLH; Calbiochem), schistosomiasis soluble egg antigen (SEA), the synthetic polypeptides (T, G)-A–L (copolymer of L-(tyrosine, glutamic acid) coupled to D,L-alanine on a poly-L-lysine backbone, batch no. MC 9) and GAT (random copolymer of 60% glutamic acid, 30% alanine and 10% tyrosine, batch no. GAT 8) (both Miles Yeda), purified protein derivative (PPD) and tetanus toxoid (both RIV). Many individuals have been knowingly sensitised to these latter two antigens; but we emphasise that all data presented here have been obtained using combinations of antigens and cells which failed to respond in a direct proliferative assay.

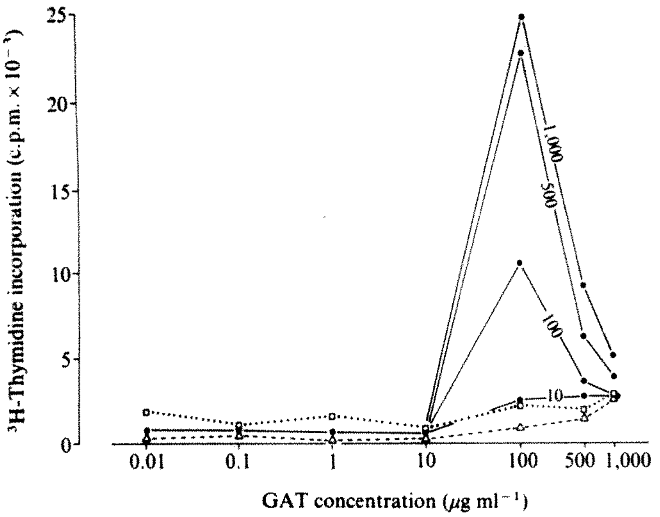

Fig. 1 Secondary responses of lymphocytes sensitised *in vitro* to GAT. Blood was obtained by venipuncture and lymphocytes were isolated by Ficoll–Hypaque density centrifugation (1.077 mg ml^{-1}). Cells from the interphase were washed 3 times in Hank's balanced salt solution and resuspended in RPMI 1640 supplemented with 30% AB serum, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin, and adjusted to 2×10^6 cells per ml. Of this cell suspension, 2.5 ml was mixed with 2.5 ml of the antigen diluted in RPMI 1640 (without serum) in a 25-cm bottle (Falcon no. 3013). Bottles were gassed with a 5% CO_2/air mixture, closed and cultured for 10 d at 37 °C in upright position. Approximately 1 in 10 of the cultures failed to retain an adequate *p*H and were discarded. Cells were then collected, adherent cells with a rubber policeman, washed once, and readjusted to 2×10^6 cells per ml in RPMI 1640 with 30% AB serum. Restimulation was carried out in duplicate or triplicate in flat-bottomed microtitre plates. A volume of 0.1 ml of the cell suspension was mixed with 0.1 ml RPMI 1640 (without serum) containing antigen. After 48 h tritiated thymidine was added, the cultures were collected 18 h later, and samples counted in a liquid scintillation counter. Seven different sensitising concentrations of GAT are shown on the abscissa. The second cultures of the sensitised lymphocytes were restimulated with: 10, 100, 500 or 1,000 μg ml^{-1} of GAT (●), 100 μg ml^{-1} of PPD (□), or medium only (△). When 10 μg ml^{-1} or less of GAT was used in the sensitising culture, all responses to GAT in the second culture were less than 1,000 c.p.m. and are not shown separately. The secondary responses of the GAT-sensitised lymphocytes to (T, G)-A--L in the concentrations 10, 100, 500 and 1,000 μg ml^{-1} are also not shown. These responses were all in the range of the responses to PPD (with a maximum of 3,200 c.p.m.). In this experiment there was no evidence for cross-reactivity between GAT and (T, G)-A--L.

To obtain *in vitro* sensitisation, we found that the most critical parameter was the concentration of antigen during the first culture. Figure 1 shows an example of results obtained, in which GAT was used as antigen. With sub-optimal low concentrations of antigen no specific proliferative response was found in the second culture. In this experiment, 100 μg ml^{-1} GAT yielded optimal specific sensitisation, as shown by the highly significant response in the second culture to GAT compared with that to other antigens. Higher concentrations showed low, or even depressed, proliferative responses in the second culture, depending on the antigen used. In general, the background proliferative responses, that is, the responses to unrelated or no antigen in the second culture, increased with concentrations of antigen slightly higher than the optimal concentration in the first culture. The nature of this phenomenon is now being investigated. Finally, the concentration of antigen used in the second culture had to be varied but was less critical. The optimal concentration of antigen for the second culture has always been greater than the optimal sensitising concentration (Figs 1, 2). For sensitising cultures with PPD and tetanus toxoid, the optimal antigen concentrations were less than 1/10th of the optimal concentrations routinely used by us for the direct lymphocyte transformation test with *in vivo* sensitised cells.

Figure 2 gives the results of an experiment in which cells were separately sensitised to three different antigens and restimulated with each of the three antigens. The data used were obtained with the optimal concentrations of each antigen. For all three antigens the proliferative response was significantly increased ($P < 0.01$, by analysis of variance on log transformed data) if the same antigen was used in the first as well as in the second culture. The lymphocytes in this and similar experiments were all negative in the direct lymphocyte transformation test with these antigens. Only the absence of current immunity can be concluded from a negative direct lymphocyte transformation test, and especially for tetanus toxoid a negative direct test does not exclude prior *in vivo* sensitisation. In further experiments, we used other antigens including synthetic polypeptides, for which it is much less likely that individuals have been sensitised *in vivo*. Table 1 shows the results of such experiments. In each experiment, at least two different antigens were used at several concentrations in the sensitising cultures. The lymphocytes from each culture were then restimulated with the relevant antigens. It was again shown that a specific proliferative response was only obtained using the same antigen in the second culture as had been used in the sensitising culture. Each experiment has been repeated at least three times with different donors and demonstrates our ability to sensitise unprimed human lymphocytes *in vitro* to different protein antigens.

We thus find that we can consistently sensitise lymphocytes from many different individuals to the antigens KLH, GAT, PPD and tetanus toxoid. Of 14 individuals tested with (T, G)-A--L and GAT, all responded to GAT whereas three failed to respond to (T, G)-A--L. These three also failed to show cross-sensitisation by GAT for response to (T, G)-A--L in the second culture, whereas 9 out of the other 11 showed such a reaction. For one of the non-responders the test has been repeated three times with different bleedings showing the same results. The genetic basis of this unresponsiveness to (T, G)-A--L is not known but is now under investigation. Restimulation after

Table 1 Proliferative response of human lymphocytes to restimulation *in vitro*

Antigen concentration (μg ml^{-1})		
First culture	Second culture	Response in c.p.m. per 10^5 cells ± s.d.
KLH 1	KLH 100	*41,163 ± 823
	KLH 500	*40,311 ± 4,434
	SEA 100	6,232 ± 1,495
	SEA 500	6,835 ± 273
	—	6,413 ± 1,796
SEA 1	KLH 100	2,442 ± 170
	KLH 500	2,044 ± 141
	SEA 100	*5,234 ± 969
	SEA 500	*5,089 ± 1,007
	—	1,907 ± 303
(T, G)-A--L 500	(T, G)-A--L 500	*30,709 ± 5,527
	(T, G)-A--L 1,000	*31,651 ± 158
	GAT 500	18,177 ± 908
	GAT 1,000	19,614 ± 2,549
	—	7,302 ± 1,095
GAT 100	(T, G)-A--L 500	21,941 ± 1,316
	(T, G)-A--L 1,000	20,072 ± 1,003
	GAT 500	*33,528 ± 3,017
	GAT 1,000	*51,376 ± 2,055
	—	14,128 ± 2,684

Lymphocytes from one donor were cultured with different antigens in the first culture and restimulated as indicated. The results show the mean and standard deviation of duplicates. In this particular experiment, cross-reactivity between (T, G)-A--L and GAT which was found in nine out of eleven cases, is also shown. Each experiment was repeated at least three times with different donors and showed the same pattern of results.

* Results significantly different from controls ($P < 0.01$).

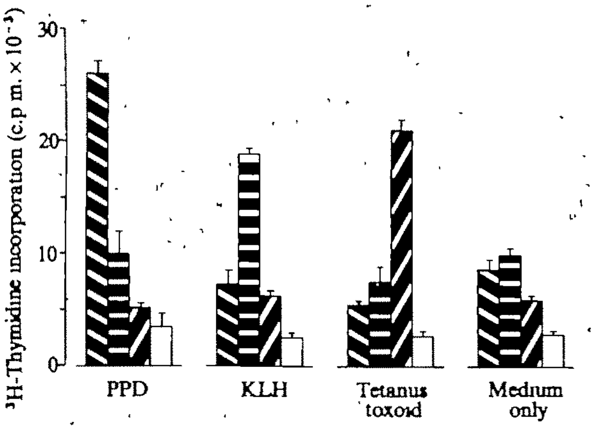

Fig. 2 Secondary responses of lymphocytes sensitised *in vitro* to PPD, KLH and tetanus toxoid. Lymphocytes were sensitised to four different concentrations of the antigens. Only data from the optimal sensitising concentrations are shown. These were 0 2 μg ml^{-1} of PPD (▧), 0 2 μg ml^{-1} of KLH (▤), 10^{-4} Lf ml^{-1} of tetanus toxoid (▨) and medium alone (☐) The optimal concentrations of antigen for restimulation were 50 μg ml^{-1} of PPD; 100 μg ml^{-1} of KLH or 10^{-1} Lf ml^{-1} of tetanus toxoid. In this experiment, the background proliferation in the second cultures was not influenced by the concentration of the irrelevant antigens. Second cultures were carried out in duplicate and results are shown ± s d. Antigens used for restimulation are indicated on the abscissa. Antigens used for sensitisation are shown by the hatching of the bars.

sensitisation with SEA was not specific in 50% of the cases, possibly because this material is a complex of many antigens. It should be added that this culture method can also be used for sensitisation to insoluble antigens, and a study of the *in vitro* response to TNP-modified autologous cells is under way.

In vitro sensitisation to soluble antigens has been previously described by Thomas and Shevach[4] using guinea pig cells. However, in their cultures, macrophages obtained from oil-induced peritoneal exudate cells were present. Comparable culture conditions for human cells would be difficult to obtain. An alternative assay for *in vitro* sensitisation, a primary *in vitro* antibody response, should be mentioned here. This method was described in humans by Dosch and Gelfund[5], and elaborated to a further extent by UytdeHaag *et al.*[6]. Comparison of the two methods is difficult because lymphocyte proliferation has not been reported in their investigations, and we have not determined whether our cultures contain antibody-secreting cells.

Therefore, our data demonstrate that specific proliferative responses to soluble protein antigens can be induced *in vitro* using peripheral blood lymphocytes from donors who had not been immunised *in vivo* against these antigens. It is hoped that this assay will lead to a further understanding of the control of the immune response in man, and in particular the analysis of human immune response genes.

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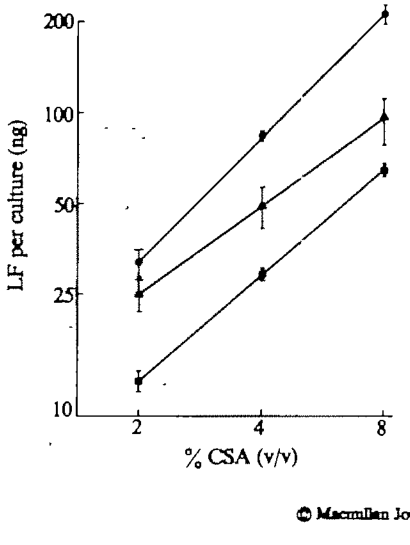
Immunochemical quantification of *in vitro* neutrophilic granulocyte differentiation

IN the presence of colony stimulating activity (CSA)[1-5], both normal and neoplastic cells of the neutrophilic granulocyte (neutrophil) and monocyte–macrophage cell series proliferate and differentiate in semi-solid and liquid cultures[6-9]. However, as conventionally performed, the clonal assay for CSA depends on cellular proliferation, and does not provide quantitative information on the role of CSA in differentiation. Lactoferrin (LF) is a specific protein marker for the neutrophil cell series[10-12], and among bone marrow cells is found exclusively in the specific granules of the maturing neutrophil[13,14]. These granules first appear at the myelocyte stage of cytodifferentiation[15]. Thus, synthesis of LF is a biochemical event which may be used to quantify a specific state of neutrophil differentiation. We previously reported the purification of murine LF[16], and its quantification by radioimmunoassay[17], and also the development of a slide chamber method for culturing bone marrow cells in liquid medium[18]. We now report that in similar cultures, the net synthesis of LF is correlated in a dose-dependent manner with CSA concentration. To our knowledge, this is the first demonstration of a quantitative relationship at both the biochemical and cellular levels between CSA and a specific marker of neutrophilic granulocyte differentiation[19].

Fig. 1 Dose–response relationship between the net synthesis of lactoferrin and the concentration of CSA in cultures of mouse bone marrow cells. Cells were obtained from 4-month-old male BALB/c mice from our breeding colony A single cell suspension in Hank's balanced salt solution deficient in calcium and magnesium was layered over a solution of Ficoll–Hypaque (density 1.077 g cm^{-3}), and centrifuged at room temperature for 30 min at 400 g. Cells collecting at the interface were resuspended in RPMI-1640 culture medium containing 20% fetal bovine serum, and used to initiate cultures (1 ml, 1.25 × 10^4 cells) in four-chamber Lab-Tek microscope slide chambers[18]. Cultures were stimulated at time zero by the addition of various amounts of CSA from endotoxin-treated mouse lung[20], and were incubated at 37 °C in a fully humidified atmosphere containing 7.5% CO_2. Cultures were terminated at various times by carefully drawing off the medium and adding 0.5 ml of 0.01 M phosphate buffered saline, *p*H 7.4, containing 0.1% Triton X-100 and 0.3 M sodium chloride. The mixture was allowed to stand for 20 min at 4 °C after which it was removed by aspiration. Aliquots of the aspirate were taken for quantification of LF by radioimmunoassay[17]. With the low initial cell inoculum used in these particular experiments (where correlation with microscopic observations was important) levels of LF on days 1 and 2 of culture were not sufficiently high to be detected by the radioimmunoassay. Each point is the mean of two to three replicates. The vertical bars indicate the range of values on day 3 (■), day 4 (▲), and day 5 (●).



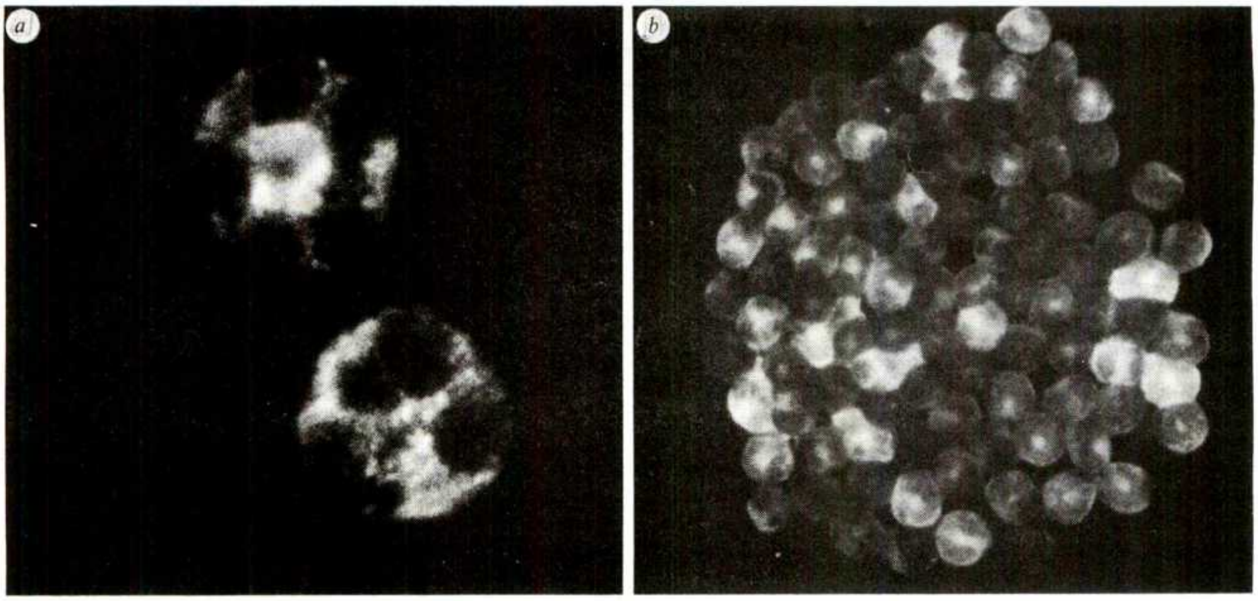


Fig. 2 Immunocytofluorescence of LF-containing, cultured murine bone marrow cells. After careful removal of the culture medium, wet slides were immediately centrifuged in a cytocentrifuge and cells were fixed in absolute ethanol containing 10% formalin[18]. Fixed cells were incubated with the fluorescein-labelled F(ab')$_2$ fragment of rabbit IgG raised against murine LF[16]. Slides were viewed using a Leitz Orthoplan fluorescence microscope equipped with a 100-W mercury lamp and epi-illumination. Photographs were taken with a Leitz Orthomat camera and Kodak Ectachrome ET135 film. *a*, Mature neutrophils showing a pattern of fluorescence characteristic of cells which have differentiated to the myelocyte stage or beyond (×1600); *b*, a single neutrophilic granulocyte clone displaying asynchronous differentiation as indicated by the presence of both LF-positive and LF-negative cells (×400).

Slide chamber cultures of murine bone marrow cells were stimulated by the addition of CSA from endotoxin-treated mouse lung[20]. When examined between days 3 and 5, a linear dose–response relationship was observed between dose of CSA and the total amount of LF per culture (Fig. 1). In addition, it was evident from these data that the magnitude of the response between day 3 and day 5 increased approximately threefold as CSA concentration increased from 4 to 8%.

It was possible to extend these biochemical observations to the cellular level by examining cultured cells which had been fixed and exposed to a fluorescein-conjugated anti-LF antibody. LF-containing cells displayed a characteristic granular pattern of fluorescence (Fig. 2*a*). As shown in Table 1, there was a linear dose–response relationship between dose of CSA, the number of LF-containing cells, and the total number of cells per culture. The latter response (total cells) was more pronounced because the marrow cell population contained precursor cells which proliferated in response to CSA, but whose progeny did not contain LF (neutrophils less mature than myelocytes, and all cells of the monocyte–macrophage series).

The distinctive growth pattern of neutrophils offered an additional advantage for immunocytofluorescence studies. In the liquid cultures, clones of neutrophils grew as discrete, tight, spheroidal cell groupings which could be easily identified through the first 3 days of culture. In these early cultures, we observed that within individual clones, the proportion of cells which contained LF varied from zero to unity, with some clones consisting of both LF positive and LF negative cells (Fig. 2*b*). This pattern of asynchronous differentiation within individual clones has also been observed in both normal and leukemic *in vitro* erythropoiesis[21,22]. Note that with the cell concentrations used in the present experiments, cell crowding made interpretation of clonally-related events difficult beyond day 3 of culture. However, use of a lower initial cell inoculum (5 to 10×10^2 cells) resulted in sufficient spatial separation of individual clones to permit observation of clonally-related events beyond day 3. In these conditions, we observed that as tight clones matured, all cells eventually contained LF[23]. We are currently investigating the effects of CSA concentration on the time course of LF synthesis in these clones composed exclusively of cells committed to neutrophilic granulopoiesis.

Table 1 Effect of increasing concentration of CSA on the total number of LF-containing cells and on the total number of cells per culture

CSA concentration (μl per ml culture)	No. of cells* LF-containing	Total
4	1,326 ± 99	3,384 ± 261
16	2,347 ± 153	5,756 ± 206
64	3,188 ± 301	8,856 ± 643

* Details of the culture and immunofluorescence procedures are as described in the legends to Figs 1 and 2 except the initial cell inoculum was 1×10^4 cells (8% of the cells contained LF). In addition to cells committed to neutrophilic granulopoiesis, the total cell population contained cells of the monocyte–macrophage series which also proliferated in response to CSA. Cultures were terminated after 3 days, and all cells in each culture chamber were counted and classified by sequential observation with phase contrast and fluorescence microscopy (×400). Each point is the mean ± s.e.m. of four chambers.

There is now good evidence that CSA preparations are heterogeneous with respect to biological activity, and that different species of CSA may govern the differentiation of neutrophilic granulocytes as distinct from monocytes and macrophages[24–27]. Furthermore, there is the possibility that some molecules of CSA might have either proliferative- or differentiative-associated functions while others might possess both of these activities. The assays for LF presented above should aid in the identification and purification of such postulated CSA species. Finally, the question as to whether CSA plays a direct part in neutrophil differentiation must remain open. The demonstration of a dose–response between CSA and net synthesis of a differentiation-associated protein such as LF is necessary but not sufficient proof of direct involvement, as the response might be secondary to an earlier, primary effect on neutrophil proliferation. However, studies on CSA-dependent synthesis of LF in the presence of appropriate inhibitors of DNA and RNA synthesis should provide further insight into this question.

In conclusion, we anticipate that the radioimmunoassay and immunocytofluorescent procedures for the detection and quantification of LF described here, both individually and in combination, will prove useful in further defining the mechanism of action of CSA in the regulation of *in vitro* granulopoiesis.

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5,6-Epoxyretinoic acid opposes the effects of 12–*O*-tetradecanoylphorbol-13-acetate in bovine lymphocytes

12-*O*-TETRADECANOYLPHORBOL-13-ACETATE (TPA), the most potent of a series of tumour-promoting agents isolated from croton oil[1], has been found to be highly active in bovine lymphocytes as a stimulator of choline phospholipid metabolism[2], an inducer of ornithine decarboxylase[3], and a co-mitogen when used in conjunction with phytohaemagglutinin (PHA)[4,5]. These effects of TPA in the bovine lymphocyte system are antagonised by retinoic acid[2,5], an agent which opposes the tumour-promoting action of phorbol esters in mouse skin[6,7] and inhibits chemical carcinogenesis in several other tissues[8]. However, the dose–response curves for retinoic acid in inhibiting these effects in lymphocytes are broad and extend over several orders of magnitude, suggesting that retinoic acid may have to be metabolised to be biologically effective in this system.

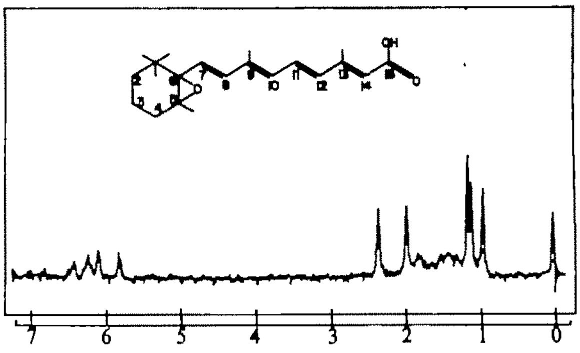

Fig. 1 The 60 MHz proton magnetic resonance spectrum of 5,6-epoxyretinoic acid. $DCCl_3$ containing 1% tetramethylsilane as internal standard was the solvent used in recording the above spectrum: δ (p p.m.) 5.77–7.08 (6H, m, olefinic protons on side chain); 2.33 (3H, s, H_3C- on C-9); 1.97 (3H, s, H_3C- on C-13); 1.2–1.9 (6H, m, $-CH_2-$ in ring); 1.13, 1.08 (6H, 2s, H_3C- on C-1); 0.92 (3H, s, H_3C- on C-5). This spectrum agrees with data presented by Vetter *et al.*[11] and is very similar to the spectrum reported for 5,6-epoxyretinal (ref. 12). The mass spectrum of 5,6-epoxyretinoic acid (melting point 153–154 °C) contained an intense molecular ion (M, *m/e* = 316) and M+1 and M+2 peaks with intensities of 21% M and 2% M, respectively. No ions of higher *m/e* were observed. The UV absorption maximum found for this material (337 nm, ethanol) agrees with that reported for enzymatically produced 5,6-epoxyretinoic acid (337 nm, light petroleum)[13].

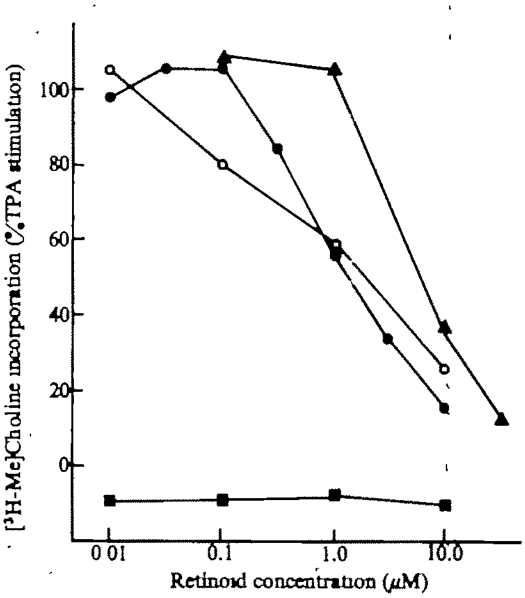

Fig. 2 Dose–response curve for the inhibition of [^{3}H-Me]choline incorporation into the phospholipids of bovine lymphocytes. Lymphocytes were prepared from bovine retropharyngeal lymph nodes as described previously[4], and were cultured in Eagle's HeLa medium supplemented with 10% bovine serum (BEHM) at a concentration of 5–10 × 10^6 cells ml^{-1} for 1 d before use. Cells were collected by centrifugation at 150*g* for 10 min and resuspended in Dulbecco's phosphate-buffered saline containing 5.6 mM glucose at 3 × 10^6 cells ml^{-1}. Five-ml portions of cell suspension were then planted in 12-ml conical centrifuge tubes containing retinoic acid or the epoxide; after 15 min at 37 °C, 2 μCi of [^{3}H-Me]choline (69.5 Ci $mmol^{-1}$) and 50 pmol of TPA were added to each culture. Dimethyl sulphoxide, used as the solvent for TPA and the retinoids, was present at a final concentration of 0.55% (v/v) in all cases. After a 1-h incorporation period, the cells were collected and the phospholipids extracted and purified as described elsewhere[2] Radioactivity in the dried phospholipid extracts was quantitated by liquid scintillation spectrometry. Points represent the means of duplicates. ○, TPA plus retinoic acid; ●, TPA plus 5,6-epoxyretinoic acid; ▲, TPA plus 5,6-epoxyretinoic acid with medium containing 10% bovine serum; ■, 5,6-epoxyretinoic acid without TPA.

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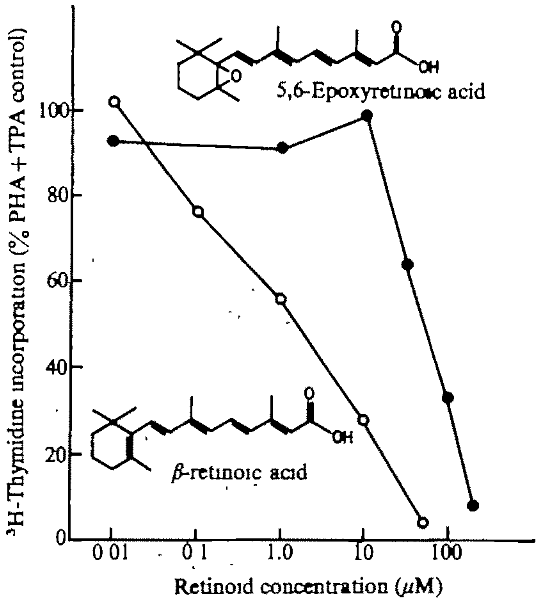

Fig. 3 Dose-response curves for the inhibition of lymphocyte mitogenesis by retinoic acid (○) and 5,6-epoxyretinoic acid (●). Cells were cultured for 1 d as described in the legend to Fig 2. The lymphocyte cell density was then adjusted to 3×10^6 cells ml^{-1} in BEHM and 5-ml aliquots of the suspension were planted in conical 12-ml centrifuge tubes. PHA-P, used at a final dilution of 1:10,000, TPA (10 nM) and the retinoids were added to cultures concurrently. The cultures were gassed with 5% CO$_2$ in air and incubated at 37 °C on a slant for 48 h The cells were then pulse-labelled for 2 h with 2 μCi per culture of ^{3}H-thymidine (64.7 Ci mmol^{-1}). The incorporated radioactivity was assayed using a standard Whatman GF/C filter disk procedure[15]. Points represent the means of triplicate cultures

The possibility that epoxidation might be involved in the activation of retinoic acid and related retinoids was suggested by two observations. First, 10,11-epoxymethylfarnesoate (insect juvenile hormone III), which bears the epoxide moiety at a position corresponding to the double bond in the ionone ring of retinoic acid, was found to oppose the actions of TPA in bovine lymphocytes and exhibited a much steeper dose-response curve[9]. Second, we have demonstrated that epoxidation at the 5,6-position of β-ionone strikingly increases its activity as an antagonist of the co-mitogenic action of TPA as well as the TPA stimulation of phospholipid metabolism[10]. We describe here the chemical synthesis and biological testing of the 5,6-epoxy derivative of retinoic acid as an antagonist of TPA action in bovine lymphocytes. We report that the introduction of an epoxide group at this position results in an enhanced ability to oppose certain effects of TPA in these cells.

Retinoic acid (2 mmol) was epoxidated by treatment with a 10-fold molar excess of monoperphthalic acid in ethyl ether (30 ml) at room temperature, and the reaction was monitored by thin layer chromatography on silica gel, with 20% ethanol in light petroleum (boiling point 40–60 °C) as the eluant. Within 0.5 h, all the retinoic acid had been consumed, with the formation of a single more polar product, as shown by thin layer chromatography. This reaction mixture was extracted twice with two volumes of aqueous NaHCO$_3$ (2% w/v) and washed with two volumes of water. When the organic phase was taken to dryness under vacuum, the product crystallised spontaneously (1.7 mmol); the latter was recrystallised from ethyl ether/light petroleum and characterised as 5,6-epoxyretinoic acid. The structure verification of racemic 5,6-epoxyretinoic acid is presented in Fig. 1.

As noted above, TPA accelerates the incorporation of choline into the phospholipids of bovine lymphocytes, and this response is antagonised by retinoic acid[2]. Figure 2 shows that 5,6-epoxyretinoic acid is also an active antagonist of this process and that when an effective concentration has been reached it exhibits a steeper dose-response curve than retinoic acid. Although other explanations of the data are possible, this observation is consistent with the hypothesis that epoxidation of the 5,6-double bond may be involved in the metabolic activation of retinoic acid. This possibility is strengthened by the report by Napoli *et al.*[14] that 5,6-epoxyretinoic acid is a major retinoic acid metabolite in the intestinal mucosa of the rat.

Several additional points should be made concerning the data presented in Fig. 2. First, it seems unlikely that the inhibitory action of the epoxide is merely a manifestation of toxicity, as it does not affect the basal level of [^{3}H-Me]choline incorporation, and second, when 10% serum was included in the incubation medium the effective inhibitory range of epoxide concentrations was shifted upward by an order of magnitude. This observation suggests that serum and certain cellular components may sequester or facilitate the inactivation of low levels of 5,6-epoxyretinoic acid and thus account for the shoulder on the dose-response curve. This same situation was encountered in earlier experiments in which a similar serum effect on the effective inhibitory concentrations of 5,6-epoxy-β-ionone was observed[10].

In Fig. 3, the contrast of broad- compared with steep-sloped dose-effectiveness curves for retinoic acid and the epoxide is clearly shown in the inhibition of the mitogenic response. As reported previously, retinoic acid inhibits TPA-mediated co-mitogenesis over a broad range of concentrations[5]; however, it

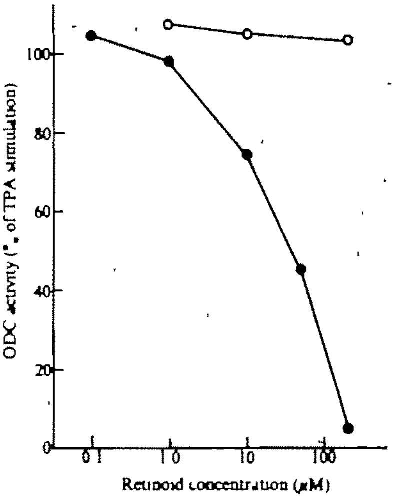

Fig. 4 Dose-response curves for the inhibition of ornithine decarboxylase (ODC) induction by retinoic acid (○) and 5,6-epoxyretinoic acid (●). Lymphocyte cultures were planted as described in the legend to Fig. 3. Eighteen hours after the concurrent addition of PHA, TPA, and retinoids to the cultures, the cells were collected and washed twice with cold 0.9% NaCl. The lymphocytes were then suspended in 0.3 ml of 50 mM sodium phosphate buffer (*p*H 7.2) containing 0.1 mM EDTA, and disrupted by four cycles of freezing and thawing. After centrifugation of the disrupted cells at 2,000*g* for 15 min, a clear supernatant was obtained. Ornithine decarboxylase activity in this supernatant was assayed by measuring the release of ^{14}CO$_2$ from DL-[1-^{14}C]ornithine hydrochloride (49.9 mCi mmol^{-1}). The assay mixture contained 35 mM sodium phosphate (*p*H 7.2), 0.2 mM pyridoxal phosphate, 4 mM dithiothreitol, 1 mM EDTA, 0.4 mM L-ornithine containing 0.5 μCi of DL-[1-^{14}C]ornithine hydrochloride, and 100 μl of lymphocyte supernatant in a total volume of 0.25 ml. Incubation of the assay mixture at 37 °C was carried out in 15-ml corex tubes equipped with centre well assemblies and capped with serum stoppers After 1 h, the reaction was stopped by the addition of 0.5 ml of 2 M citric acid, however, incubation was continued for one more hour to ensure complete absorption of ^{14}CO$_2$ by the ethanolamine and methoxyethanol (0.2 ml; 2:1) in the centre well. Finally, the contents of the centre well were transferred to a vial containing 10 ml of toluene-based scintillation fluid and 2 ml ethanol. Radioactivity was measured by liquid scintillation spectrometry. Points represent the means of triplicate cultures.

can be seen that retinoic acid epoxide displays a steep dose–response curve, as anticipated for the active inhibitory species. The fact that higher concentrations of the epoxide, as compared with the parent compound, are required to achieve inhibition is thought to reflect the above noted difficulty in delivering the chemically reactive epoxide to the appropriate cellular sites rather than an inherently lower activity of this derivative. Again, toxicity does not seem to be the basis of this inhibitory action of 5,6-epoxyretinoic acid, for 48 h of exposure to this agent does not affect the lymphocyte viability, as measured by the trypan blue exclusion test.

In a third series of studies, we have tested the ability of 5,6-epoxyretinoic acid to inhibit the induction of ornithine decarboxylase by TPA. Ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis, is among the earliest and most thoroughly studied responses induced by the application of TPA to mouse skin[16]. The TPA induction of this enzyme, which is prevented by retinoic acid, has provided a useful test system for assessing several retinoid analogues as biological antagonists of TPA in mouse skin. In PHA-treated lymphocytes, retinoic acid also blocks the TPA-induced ornithine decarboxylase activity; however, it must be added to cell cultures before TPA to exert this effect[3]. This temporal dependency is reflected in Fig. 4, in which the induction of ornithine decarboxylase by TPA proceeds unhindered by concurrently added retinoic acid. However, in these same conditions, 5,6-epoxyretinoic acid is a highly effective inhibitor of ornithine decarboxylase induction (Fig. 4).

The present results do not prove the concept of metabolic activation of retinoic acid, but they are consistent with the suggestion that an activation process may involve epoxidation at the 5,6-position. At the very minimum, it can be stated that epoxidation of retinoic acid at the 5,6-position is a tolerated modification of retinoic acid for activity of this agent as an antagonist of certain effects of TPA in the bovine lymphocyte system.

Thus, although bovine lymphocytes have not been used in tumour-promoting studies with phorbol esters or in anti-tumourigenic studies with retinoids, the present results and our earlier findings[2–5,9,10] clearly demonstrate that they are a good indicator system for studying the modulation of metabolic processes by these two classes of agents. The responsiveness, the ready availability of the cells, and the ease of staging prompts their further use in studies of the molecular mechanisms by which these agents act.

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Migrating epidermis produces AB$_2$ collagen and requires continual collagen synthesis for movement

EPITHELIAL CELLS demonstrate remarkable motility during the *in vivo* processes of wound healing, organogenesis and neoplastic invasion[1]. We have been interested in the control of epithelial motility. From earlier studies we know that *in vitro* epithelial movement requires the continual presence of a serum component[2,3] and a proper substratum[4]. In culturing dissociated epithelial cells, collagen-coated dishes have been found to provide a better substratum than non-coated surfaces[5]. As studies in other laboratories had suggested that epithelium produces collagen[6–8], it seemed appropriate to investigate the role of collagen in epithelial movement. Reported here are morphological and metabolic studies of a well defined tissue culture system of skin explants involving both epithelial outgrowth and epiboly. The observations support the possibility that the continual synthesis of collagen is necessary for epithelial cell movement.

The *in vitro* system we have used in measuring epithelial movement has been described elsewhere in detail[2,3]. Briefly, measured mouse skin explants are laid on plastic Petri dishes (outgrowth) and covered with medium (Dulbecco's modified Eagle's medium, DME; Gibco) plus human serum (5 mg ml^{-1}) or suspended in the medium (epiboly). At intervals, the maximal radial outgrowth or the extent of epiboly are measured. In earlier experiments we established that epithelial spread is virtually independent of DNA synthesis and thus cell division; the outgrowth measured is therefore apparently due predominantly to epithelial cell motility. Although the explant contains dermal elements the outgrowth is essentially, if not completely, epithelial, as demonstrated earlier[2,3].

Our initial questions were: does migrating epithelium of the outgrowth and epiboly systems contain collagen? If so, what types of collagen are present in the migrating epidermis? Using affinity-purified antibodies to collagen (I, III, IV and AB$_2$) and an indirect immunofluorescence (IF) assay, we found that both stationary and migrating epidermis contain type AB$_2$ collagen (Fig. 1i, 1j). Types I (Fig. 1c, 1d) and III (Fig. 1e, 1f) are found exclusively in the dermal portion of the outgrowth. Type IV (Fig. 1g, 1h) is localised to the junction of the dermis and the stationary epidermis, but not beneath the migrating epidermis.

If both migrating and stationary epidermis synthesise AB$_2$ collagen, as the IF studies suggest, proline incorporation into these cells should be demonstrable, as proline and hydroxyproline constitute 20% of the amino acid residues of AB$_2$ collagen[9]. The autoradiography of ^{3}H-proline-pulsed explants (Fig. 1a, 1b) shows silver grains over the cytoplasm of the migrating and stationary epidermis of both the outgrowth and the epiboly assays. Proline incorporation was present diffusely over the epithelial cell cytoplasm and grain density was not greater at the growing tip or the basement membrane regions. As essentially no proline incorporation was present in the dermis at any time throughout the experiment, we assume that proline is incorporated by the epithelium and not transferred to the epithelium from the dermis.

The synthesis of collagen by the epithelium was tested first by hydroxyproline synthesis and incorporation (a product of

Table 1 Epithelial spreading in the presence of the proline analogue L-azetidine-2-carboxylic acid

Experimental conditions	Linear outgrowth at 3 d (μm)	% Of control	Epiboly (%)
DME + HS (control)	465 ± 34 (35)	100	96 ± 4 (9)
DME + HS + cycloheximide (1 μg ml^{-1})	0 ± 0 (5)	0	Not done
DME + HS + cycloheximide (5 μg ml^{-1})	0 ± 0 (12)	0	Not done
DME + HS + LACA (1 μg ml^{-1})	527 ± 65 (6)	113	Not done
DME + HS + LACA (10 μg ml^{-1})	48 ± 15 (32)	10	5 ± 1 (11)
DME + HS + LACA (100 μg ml^{-1})	0 ± 0 (11)	0	0 ± 0 (9)
DME + HS + LACA (10 μg ml^{-1}) for 1 d and then DME + HS for 2 d	273 ± 33 (10)	113*	Not done
DME + HS + proline (200 μg ml^{-1})	459 ± 31 (16)	99	100 ± 0 (12)
DME + HS + LACA (10 μg ml^{-1}) + proline (200 μg ml^{-1})	442 ± 52 (12)	95	95 ± 4 (11)
DME + HS + LACA (100 μg ml^{-1}) + proline (200 μg ml^{-1})	254 ± 27(13)	58	89 ± 6 (8)

Outgrowth and epiboly experiments were set up as previously described[2,3]. Linear outgrowth is recorded as the maximal radial spread of epithelium measured from the explant edge. The data are recorded as the mean ± s.e.m. (no. of observations). Outgrowth in the presence of DME + HS for 3 d was used as the control in calculating the percent of outgrowth except for the recovery experiment (indicated by asterisk), where outgrowth after 2 d in DME + HS was used as the control. Epiboly is expressed as the mean per cent of the lower explant covered by epithelium ± s.e.m. (no. of observations) after 2 d of incubation. HS was prepared as previously described[2,3].

collagen biosynthesis), and second by incorporation of ^{3}H-proline into antigen precipitable by monospecific collagen antibodies. The synthesis of hydroxyproline by the epidermis was tested using epiboly explants incubated for 24 h in the conditions described in the legend to Fig. 1; however, in these experiments the ^{3}H-proline pulse was given for 24 h. The epidermis was removed from the dermis after incubating in hot water (55 °C) by the method of Marrs and Voorhees[10]. The epidermis was then hydrolysed and the hydrolysate analysed by the thin-layer chromatographic technique of Myhill and Jackson[11]. Approximately 4% of the applied counts comigrated with the hydroxyproline standard (H6002 Sigma).

To test for the incorporation of proline into collagen antigen, ^{3}H-proline-pulsed explants (grown as described in the legend to Fig. 1) were homogenised and extracted in 0.1 M acetic acid (24 h at 4 °C). The supernatant was dialysed (0.01 M acetic acid) and lyophilised. The dried collagen was suspended (0.01 M acetic acid to 100 μg ml^{-1}) and dialysed into neutral buffer (0.05 M Tris-HCl, pH 7.5, 0.15 M NaCl). To 0.2 ml of this solution, 0.1-ml aliquots of AB$_2$ collagen antibody (or normal

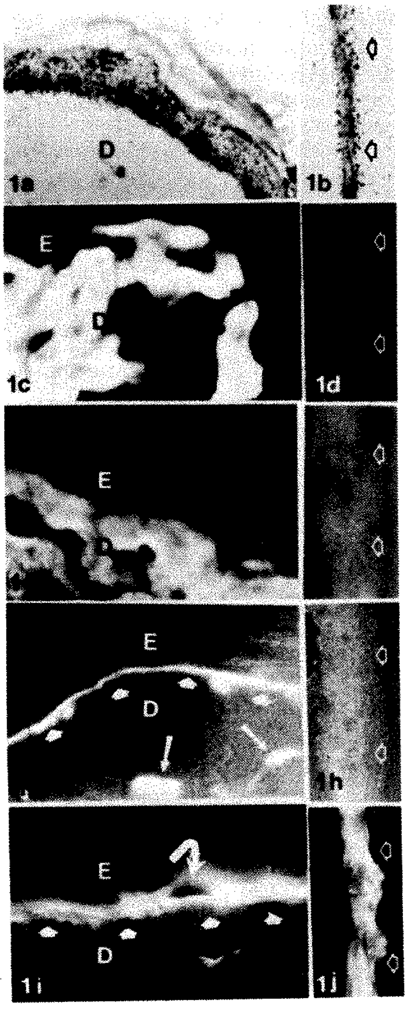

Fig. 1 Autoradiography and collagen immunofluorescence of epithelial outgrowth and epiboly. Using our previously described *in vitro* explant system[2,3], 0.2-cm disks of 6–10-week-old mouse ear skin were grown for 3 d on 0.22 μm sterile Millipore filters in DME containing 5 mg ml^{-1} dialysed and lyophilised human serum in 95% air, 5% CO$_2$ and saturated water at 37 °C. By 3 d appreciable epithelial outgrowth had extended from the explant over the filter. For the epiboly experiments, using the same tissue culture set-up, skin disks were floated on identical medium for 48 h. For both sets of experiments, at the indicated times the tissues were removed, fixed in 10% buffered formalin and prepared for light histological examination as previously described[2,3]. Autoradiography was carried out as described elsewhere[33]; the explants were pulsed for 2 h in medium containing L-[2,3^{3}H]proline (3 μCi ml^{-1}) (NEN, specific activity 20 Ci mmol^{-1}). Types I and III collagen were isolated from rat skin and types I, III and AB$_2$ collagen prepared from human placental membranes[9] by previously described methods. Type IV collagen was prepared from EHS sarcoma[36]. The collagens were judged pure by SDS-polyacrylamide gel electrophoresis of α chains (5%), CNBr peptides (7.5%) and amino acid composition. Rabbit antibodies were purified by cross-absorption on Sepharose affinity columns containing all other collagen types, followed by absorption affinity chromatography on a column containing the collagen type against which the serum was raised. They were of high titre (1 : 25,000 to 1 : 100,000) and specific for the collagen against which they were raised, as tested by haemagglutination, haemagglutination inhibition and/or radioimmunoassay[24,37–40]. Immunofluorescence (IF) staining and microscopy of frozen sections of outgrowth and epiboly tissues were done using standard techniques with fluorescein-labelled sheep anti-rabbit IgG. Immunological cross-reactivity between mouse and rat collagens (I and III) and between human and mouse collagen (AB$_2$) was demonstrated by tissue immunofluorescence and haemagglutination inhibition (type I and AB$_2$) and radioimmune assay (type III). 1a, Autoradiography of outgrowth, epithelium over dermal plug (magnification ×500); b, autoradiography of epithelial outgrowth (magnification ×500). IF using antitype I collagen antibody of skin disk (1c) (the centrally lying non-fluorescent structure is a hair follicle) (magnification ×500), and epithelial outgrowth (1d) (magnification ×500). IF using anti-type III collagen antibody of skin disk (1e) (magnification ×500) and epithelial outgrowth (1f) (magnification ×500). IF using anti-type IV collagen antibody of skin disk (1g) (magnification ×250) and epithelial outgrowth (1h) (magnification ×500). IF using anti-type AB$_2$ collagen antibody of skin disk (1i) (here the label E is above the epidermis which fluoresces at all levels) (magnification ×500), and epithelial outgrowth (1j) (magnification ×500). E, Epidermis; D, dermis; arrowheads, epithelial basement membrane; arrows, skin appendages; curved arrow, epithelial cell nucleus; open arrowheads, epithelial outgrowth. All fluorescence could be specifically inhibited by preincubation of antibody with 2.5 μg of the collagen being assayed but not by addition of other collagens.

rabbit serum) were added and the solution then incubated for 24 h (4 °C). At this time 0.2 ml of sheep anti-rabbit IgG antiserum was added followed by an additional 24 h incubation (4 °C). The AB_2 collagen antibody precipitated 15% of the acetic acid-extractable counts.

If migrating epithelium produces collagen, as the above studies suggest, is its continual synthesis necessary for epithelial movement? Inhibition studies testing this possibility are described in Table 1. No outgrowth occurred in the presence of the general protein synthesis inhibitor, cycloheximide, at concentrations of 1 and 5 μg per ml of medium. This observation agrees with the epiboly experiments of Gibbins[12]. Using the proline analogue L-azetidine-2-carboxylic acid (LACA), which affects collagen production[13,14], outgrowth and epiboly were studied. At 1 $\mu g\ ml^{-1}$ LACA, no inhibition of outgrowth was found, whereas at 10 $\mu g\ ml^{-1}$ and 100 $\mu g\ ml^{-1}$, inhibition was 90% and 100%, respectively. Removal of the inhibitor after 24 h led to the complete recovery of outgrowth. Adding L-proline to the medium (200 $\mu g\ ml^{-1}$) completely reversed the inhibition at 10 $\mu g\ ml^{-1}$ of LACA and partially corrected the inhibition at 100 $\mu g\ ml^{-1}$ LACA (Table 1). These experiments support the idea that the LACA effect is competitively inhibited by L-proline and that a proline-rich protein (perhaps collagen) is necessary for movement.

It is easiest to interpret an inhibitor experiment if the inhibition can be specifically reversed. If the proline-rich protein collagen is necessary for movement, it should theoretically be possible to observe movement in the presence of LACA if collagen were added back, that is, if collagen-coated dishes were used in the presence of LACA (Fig. 2). As observed in earlier studies[2,3] on a plastic substratum, no epithelial outgrowth is seen before 24 h, but thereafter outgrowth is linear from 1 to 3 d (Fig. 2*a*, continuous line). As seen in Fig. 2*a*, in the absence of collagen, outward spreading of epithelium abruptly stopped after the addition of LACA (100 $\mu g\ ml^{-1}$) to the medium. No outgrowth was seen if LACA was added at day 1 and no increase in outgrowth was seen if it was added at day 2. On collagen-coated dishes (Fig. 2*b*) the effect is only quantitatively different. In the absence of inhibitor and presence of collagen-coated substratum (types I, III, IV and AB_2), outgrowth at 3 d is 59% greater than that on non-coated dishes (Student's t test $P < 0.005$). If LACA was added at zero time or at 2 d, no further outgrowth occurred despite the collagen substratum. Adding the inhibitor to the outgrowth experiments after the epithelium had made contact with the substratum or to the epiboly experiments at any time caused the cessation of epithelial movement. Adding the collagen back in solution (types I, III, IV or AB_2) (50–100 $\mu g\ ml^{-1}$) did not restore motility to outgrowth or epiboly experiments in the presence of LACA.

In these studies we have demonstrated: (1) that migrating epithelium contains type AB_2 collagen but not types I, III and IV; (2) that migrating epithelium synthesises and incorporates hydroxyproline and actively incorporates proline into an antigen precipitable by antibody monospecific to type AB_2 collagen; (3) that the proline analogue LACA reversibly inhibits epithelial migration. One interpretation of these results is that migrating epithelium synthesises and contains type AB_2 collagen and that collagen production (synthesis and secretion) is necessary for movement. Although it is the simplest conclusion for these results, we do not have direct chemical evidence that collagen synthesis is necessary for movement. If only the presence of collagen in the extracellular environment were sufficient to support migration, the "add-back' experiments (collagen-coated dishes and collagen-containing media) should have overcome the LACA effect. The failure to restore motility in these experiments in the presence of LACA may indicate a more complex relationship. First, it may be possible that other proline-containing proteins are necessary for movement. We think this is unlikely not only because the cells contain collagen, but also because the system is very sensitive to inhibition (10 μg of LACA is inhibitory) and therefore the necessary proteins must be proline-rich (collagen-like). Second, it is possible that moving epithelial cells require the continual production of collagen which is cell-surface-associated or substratum-coated in a specific way which we have not been able to reproduce in the add-back experiments.

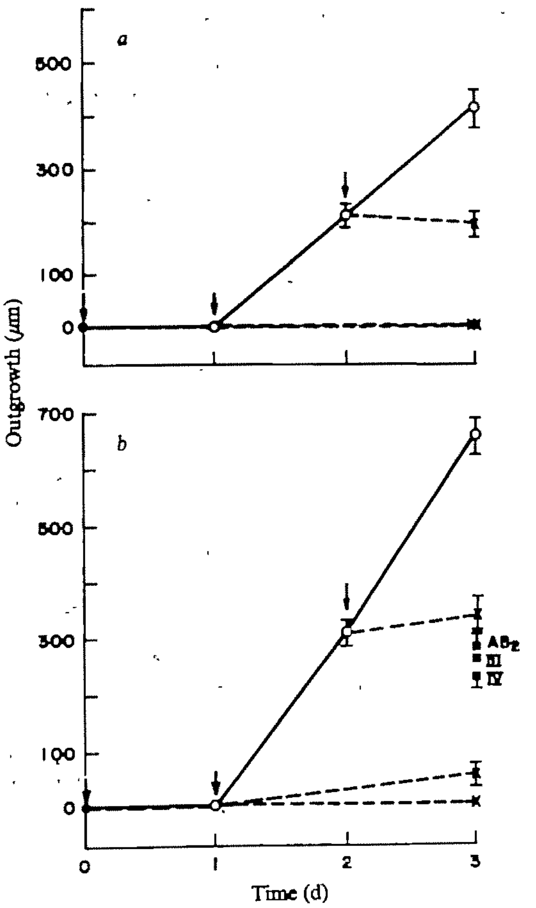

Fig. 2 Epithelial outgrowth on non-coated (*a*) and collagen-coated (*b*) dishes. As described for Fig 1 and Table 1, skin explants were laid down on Petri dishes and radial outgrowth measured over a 3-d period ○—○, DME+HS (5 $mg\ ml^{-1}$); ×---×, DME+HS (5 $mg\ ml^{-1}$)+LACA (100 $mg\ ml^{-1}$). LACA addition is indicated by arrows. Each point represents the mean of 6–14 experiments ± s.e. In *b*, the squares represent the final outgrowth after LACA (100 $\mu g\ ml^{-1}$) was added at day 2 to experiments with type III, type IV and type AB_2 collagen-coated dishes. All other points are experiments with type I-coated dishes. The dishes were prepared by the method of Karasek and Charleton[5] and the collagen concentrations were 50 $\mu g\ cm^{-2}$.

Collagen has been sought in epithelium before, although to our knowledge this is the first demonstration of type AB_2 collagen in epithelium. We were surprised that migrating epithelium did not contain type IV collagen, as epithelium is believed to produce its own basement membrane[15–20]. This observation is compatible with other studies which report that basement membrane material is not morphologically present below migrating epithelium during the initial stages of movement[15,21,22]. It is notable that AB_2 collagen has been found to be a component of some epithelial basement membranes[23,24].

LACA, a cyclic imino-acid plant extract, has been found to be competitively incorporated into proteins in place of proline[25]. Its incorporation into collagen has been demonstrated[26] and the abnormal collagens formed seem to be inadequately secreted by the synthesising cell[13,14]. The synthesis of an abnormal collagen and its inadequate secretion may explain our results in the presence of LACA. In contrast to the complete reversal by proline at low LACA concentrations (10 μg) (LACA/proline ratio of 1 : 20), only partial reversal was found at higher concentrations (100 μg) (LACA/proline ratio of 1 : 2) (Table 1). The latter case may be due to the production of abnormal collagen secondary to significant LACA incorporation[26] even in the presence of proline.

The specific role of AB_2 collagen in epithelial migration is still unknown. Other investigators have also found that collagen-coated substrata have a dramatic effect on various tissue culture systems (fibroblasts, mammary gland epithelium, hepatocytes, monocytes, corneal epithelium). The effect has included increased cellular adherence[5,27], differentiation[28,29] and protein synthesis[29]. Therefore, the observed enhancement of epithelial migration when explants are grown on collagen substrata (regardless of type) is not surprising. This phenomenon may represent a general charge or ionic effect rather than a specific property of collagen[30]. Recently, Kleinman *et al.*[31] and Murray *et al.*[32] have demonstrated in short-term cultures that dissociated epithelial cells attach preferentially to type IV collagen-coated dishes. As our studies deal with epithelial cell sheets and relatively long-term migration patterns, a meaningful comparison between these studies cannot be made.

These experiments support the hypothesis that migrating epithelium contains AB_2 collagen and must liberate this collagen for continual movement. By understanding the interaction of epithelial cells and collagen metabolism in this system, we hope to gain insight into the control of epithelial movement in particular and, perhaps, cell movement in general.

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Survival and development of ciliary ganglion neurones grown alone in cell culture

NEURONAL cell death is a conspicuous part of development for many neuronal populations in the vertebrate nervous system[1,2]. Although little is known about the mechanisms that control neurone death, it seems that interactions with the postsynaptic target tissue are important. Thus, for the chick ciliary ganglion (CG), Landmesser and Pilar showed that half of the neurones present die between days 8 and 13 in the embryo. Before cell death, all the neurones extend processes to the periphery. Ablating the periphery early in development causes more than 90% of the neurones to die at the normal time[3]. We have previously shown that nearly all the neurones from chick CG, including those destined to die *in vivo*, can survive and develop in cell culture when ganglia from 8-d-old embryos are dissociated and grown with skeletal myotubes[4]. Many of the neurones innervate myotubes in these conditions. Are neuromuscular synapses necessary to prevent the death of ciliary ganglion neurones? We report here that they are not. Culture medium conditioned by contact with heart or skeletal muscle cells permits complete long-term survival of the dissociated neurones grown in the absence of other cell types. The neurones develop levels of choline acetyltransferase (CAT) activity comparable to those obtained for neurones grown with myotubes.

Ciliary ganglia from 7–8-d-old chick embryos (Hamburger–Hamilton stages 32–34) were dissociated with trypsin as previously described[4]. The cells were plated in collagen-coated,

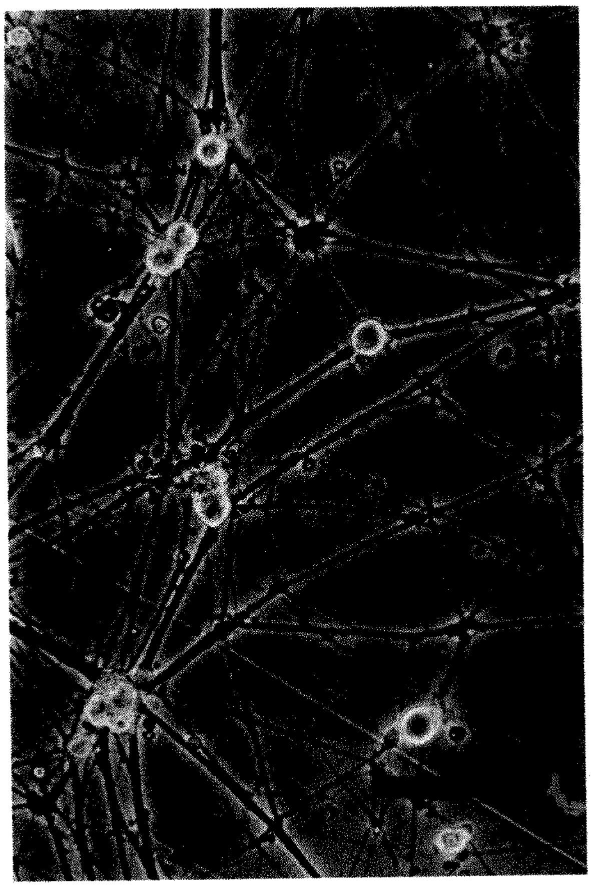

Fig. 1 Dissociated CG neurones after 3 weeks in culture. CG neurones dissociated from 8-d-old embryonic ganglia were maintained in heart-conditioned medium for 3 weeks. The photo was taken with phase contrast optics. Calibration bar: 100 μm.

Table 1 Culture medium requirements for survival of CG neurones

Culture medium	Neuronal survival (neurones per ganglion equivalent)	CAT activity (fmol ACh per neurone per h)
Conditioned + emb. ext.	6,900 ± 400	220 ± 24
Conditioned + emb. ext. + cytarabine	5,400 ± 200	ND
Unconditioned + emb. ext.	6,600 ± 100	370 ± 30
Conditioned	5,500 ± 400	170 ± 20
Unconditioned	0	ND

Cultures were seeded with ~6,500 neurones (1 ganglion equivalent) from 8-d embryos and were maintained for 11 d in the indicated medium. The cultures were then examined for surviving neurones and for CAT activity. Each value represents the mean ± s.e.m. of at least 7 cultures from 2 or more cell platings. Heart cell cultures were used to condition the medium where applicable. For these experiments only, embryo extract (emb. ext.) was not present unless indicated. Cultures treated with cytosine arabinoside (cytarabine) were exposed to a concentration of 10^{-5} M only during the first 2 d of the 11-d period. ND, not determined.

16-mm Falcon tissue culture wells at a density of 1 ganglion equivalent (~6,500 neurones) per well. Cultures plated at lower cell densities yielded variable results. Standard culture medium consisted of Eagle's minimal essential medium supplemented with 10% heat-inactivated horse serum (Microbiological Associates), 5% (v/v) chick embryo extract (prepared from 10-d-old embryos as previously described[4]), 50 μg ml^{-1} penicillin and 50 U ml^{-1} streptomycin. In our initial studies we used conditioned medium prepared by incubating standard culture medium with skeletal myotube or heart cell cultures for 72 h; the conditioned medium was passed through a Millipore filter before use.

When grown in conditioned medium, CG neurones rapidly extended processes and survived for many weeks in culture (Figs 1, 2*a*). Combining the cell counts for neurone cultures 1–3 weeks old yielded a value of 6,040 ± 160 (s.e.m. 52 cultures) for the mean number of surviving neurones per ganglion used to prepare the cultures. This represents 93% of the neurones previously shown to be present at the time of dissection[3]. Ganglionic non-neuronal cells seemed to be unnecessary for neuronal survival in these conditions, as few such cells were usually present (Fig. 1). Furthermore, 48-h exposure of the cultures to cytosine arabinoside, a drug that kills rapidly dividing cells such as ganglionic non-neuronal cells[5], only slightly reduced neuronal survival (Table 1).

Assays of CAT activity indicated that the neurones continued to develop along cholinergic lines. CG neurones grown with and without myotubes developed comparable levels of CAT activity, and the levels increased about 10-fold between days 3 and 15 in culture (Fig. 2*b*). Lactate dehydrogenase (LDH), a cytoplasmic enzyme common to many cell types, was assayed as a marker for cell cytoplasm. LDH activity increased three- to fourfold over the same period. Thus, at least a part of the increase in CAT activity represented an increase in specific activity (CAT/LDH), suggesting that neuronal development as well as net growth had occurred.

Survival of CG neurones was promoted independently by factors contributed by embryo extract in the medium and by factors derived from heart cells during the conditioning period. Unconditioned medium containing embryo extract supported complete neuronal survival and the development of high levels of CAT activity (Table 1). Nearly complete survival was also achieved in heart-conditioned medium lacking embryo extract, although lower levels of CAT activity were observed in this case. In unconditioned medium lacking embryo extract, the neurones failed to extend processes and degenerated after 1–2 d. It remains to be determined how many factors are involved and whether the heart cell factors are the same as those found in embryo extract. The differences in CAT activity per neurone observed for the various culture conditions (Table 1) raise the possibility that cholinergic development in the neurones may respond to different signals from those controlling neuronal survival. Other workers have described a factor in heart-conditioned F12 medium supplemented with 10% fetal calf serum that permitted neurite extension by CG neurones *in vitro*, but did not promote long-term survival in the conditions used[6–8].

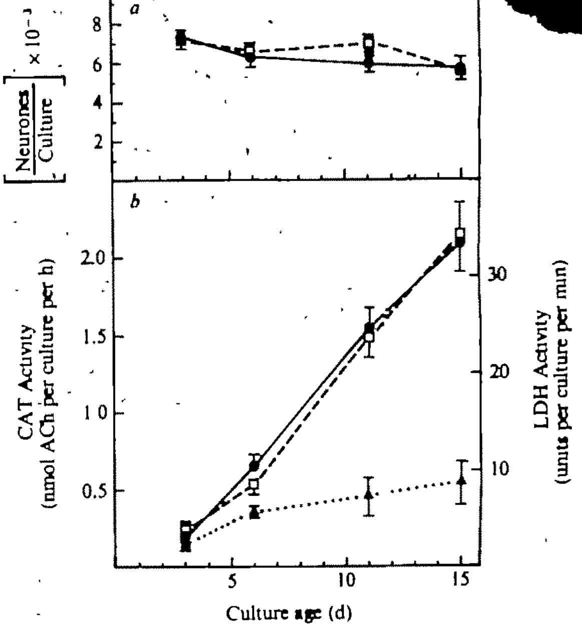

Fig. 2 Survival and development of CG neurones in culture. CG neurones from 8-d-old embryos were grown either with skeletal myotubes or alone with heart-conditioned medium. At the indicated times cultures were counted for neurones by examining 15 uniformly spaced fields of view at ×200 with phase contrast optics. Neurones were evenly distributed over the culture surface and could be distinguished by morphological criteria[4]. Such cells were previously confirmed to be electrically excitable[4] and could be autoradiographically labelled by high affinity ^{3}H-choline uptake[13]. ^{3}H-Thymidine autoradiography experiments demonstrated that CG neurones did not undergo cell division in culture (R.N. and D.K.B., unpublished observations). After counting the neurones, cultures were assayed for CAT and LDH activity as previously described[14]. Naphthyl vinyl pyridine (0.5 mM), a specific inhibitor of CAT, inhibited at least 80% of the activity. *a*, Neuronal survival; *b*, CAT and LDH activity. Neurones grown alone, □; neurones grown with myotubes, ●; LDH activity for neurones grown alone, ▲. Each point represents the mean ± s.e.m. for 7–10 cultures taken from 2–5 different cell platings. Background values for CAT and LDH activities were determined on culture wells treated identically to cell cultures except for the omission of cells; background values were always less than 10% of the culture values and were subtracted.

These results demonstrate that neither nerve–muscle synapse formation nor direct contact with muscle cells is necessary for the survival and development of CG neurones if appropriate factors are supplied in the culture medium. (The role of possible nerve–nerve synapses in such conditions[9] has not been examined.) Furthermore, it is clear that CG neurones are not irreversibly committed to cell death *in vivo*, even as late as the beginning of the normal death period. It might be argued that neurones in culture remain trapped at a developmental stage before that at which death occurs *in vivo*. We think that this is unlikely as the neurones innervated myotubes when present[4] and they developed high levels of CAT activity with or without myotubes. CAT activity on a per neurone basis at 15 d in culture was about five times greater than that reported for the sum of activity for the CG plus iris (postsynaptic target) tissue for 8-d embryos[10]. If the *in vivo* values could be corrected for contributions for preganglionic terminals, the comparison would be even vourable. CAT levels did not increase as rapidly in lture, however, as they did *in vivo*.

These findings are consistent with the suggestion that survival of parasympathetic neurones *in vivo* may be regulated in part by availability of a factor supplied by postsynaptic target cells[11]. Several lines of evidence support such a model for regulation of cell death for sympathetic neurones by nerve growth factor, although other interpretations of the data remain possible[12].

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GABA and glycine may share the same conductance channel on cultured mammalian neurones

ANALYSIS of conductance fluctuations induced during application of putative neurotransmitters to postsynaptic membranes has led to estimates of the unitary conductance and kinetics of the underlying ionic channels. Estimates have been made for a variety of receptor-coupled channels activated on invertebrate and vertebrate membranes (for review see ref. 1). Recently we have applied the technique to mouse spinal neurones grown in tissue culture and have estimated the conductance and duration of the single channel events underlying responses to the putative inhibitory transmitter γ-aminobutyric acid (GABA)[2]. We have extended our studies to another inhibitory transmitter, glycine, and report here that channels activated by glycine have properties distinctly different from those activated by GABA and that the amino acid receptors may share a conductance mechanism.

Neurones were dissociated from the spinal cords of 13–14-d-old mouse embryos and grown in tissue culture[3] until large enough to be penetrated with two microelectrodes (20–25 μM cell bodies). Recordings were made on the modified stage of an inverted microscope using phase contrast optics at ×250 magnification. GABA and glycine were applied to the cell body by microiontophoresis. $MgCl_2$ was added to a final concentration of 10 mM to suppress spontaneous synaptic activity and allow easier examination of the postsynaptic events. The temperature of the experiments was 26 ± 1 °C. When recording with either KAc or KCl microelectrodes, the inversion potential of the GABA and glycine responses are identical, averaging about −63 mV with KAc microelectrodes and about −20 mV with KCl microelectrodes[4]. This difference in inversion potential for the two recording situations is a result of increased intracellular Cl^- concentration created by Cl^- leakage from KCl microelectrodes. Prolonged amino acid responses frequently reverse polarity when recorded with KAc microelectrodes, presumably due to the time-dependent change in driving force induced by sustained activation of Cl^- conductance[4]. In contrast, KCl recordings permit a relatively more stable, albeit altered, driving force. For these reasons fluctuation experiments have been carried out using KCl microelectrodes.

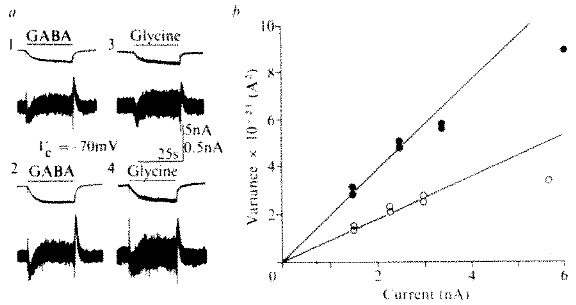

Fig. 1 GABA and glycine activate channels with different conductances on the same spinal neurone grown in cell culture. *a*, Membrane current recordings from a neurone voltage clamped to −70 mV show inward current responses during iontophoresis of GABA (1, 2) and glycine (3, 4). Iontophoresis duration is marked by bars above traces. Each pair of traces contains a d.c.-coupled trace above and a high-gain, a.c.-coupled trace below. Membrane current responses to GABA and glycine are associated with dose-dependent increases in the thickness of each trace, reflecting corresponding increases in conductance fluctuations. *b*, The variance in current fluctuation is directly related to the amplitude of the membrane current response to GABA (○) and glycine (●). Increases in variance obtained during the two sets of responses shown in *a* were computed along with those observed during two other amino acid responses and these plotted as a function of membrane current response. The first three points are approximated by straight lines whose slopes give estimates of single channel conductances of 16.4 pS and 36.4 pS for GABA and glycine, respectively. See text for explanation of deviations of the fourth points from the straight line.

Cells were voltage clamped so that the difference between the holding potential and the inversion potential for the amino acid responses (the driving force) was about 60 mV. Both amino acids caused a dose-dependent increase in inward current which was associated with a dose-dependent increase in the fluctuations of the current signal (Fig. 1). Amino acid-induced increase in variance was calculated by subtracting the baseline variance obtained before or after the iontophoretic application. This particular fluctuation analysis requires three basic assumptions: (1) the variance is derived from a statistical variation in the number of open ion channels activated by the amino acids about a mean level of open channels; (2) each ion channel event is rectangular and constant in amplitude; and (3) the probability of any channel being open is small[1]. With these assumptions an estimate of the single channel conductance, γ, can be made from measurement of the agonist-induced variance, σ^2, using the equation

$$\gamma = \sigma^2/(\Delta I(V_c - E_r))$$

where ΔI is the agonist-induced membrane current, V_c is the potential to which the cell is voltage-clamped and E_r is the reversal potential of the current response. Glycine current responses were associated with a larger variance than GABA current responses of similar amplitude. The first three points of both series of amino acid responses have been approximated by straight lines whose slopes give γ values of 15 and 29 pS for GABA and glycine, respectively. The fourth points fall below the straight lines, indicating an apparent decrease in γ. Both of the largest current responses were partially desensitised. The apparent decrease may reflect a large increase in the probability of non-desensitised channels being open, contrary to one of the assumptions on which fluctuation analysis is predicted. Estimates of γ were made for GABA and glycine applied to the

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same spinal neurones ($n=4$) and to different spinal neurones ($n=12$ cells for GABA and 6 cells for glycine). When the amino acids were compared on the same neurone γ estimates were (mean ± s.d.) 14.7 ± 3 and 30.4 ± 8.4 pS for GABA- (71 observations) and glycine-activated channels (50 observations), respectively. Estimates made on separate cells were 18 ± 8.2 pS for GABA (271 observations) and 30.4 ± 9.5 pS for glycine-activated channels (71 observations).

Channel duration estimates were made by performing spectral analyses of baseline and amino acid-induced fluctuations and then subtracting the former from the latter power density spectra. Power density spectra were calculated as the Fourier transform of at least 8,192 point samples of the fluctuations digitised at 1, 2 or 5 ms per point[5]. Spectra of GABA- and glycine-induced fluctuations could usually be approximated by a single lorentzian of the form $S(f)/S(0)=1/[1+(f/f_c)^2]$, where $S(f)$ is the spectral density as a function of frequency, f, and f_c is the frequency where the spectral density has decreased to half the value of the zero-frequency asymptote, $S(0)$, of the spectrum. Such an approximation is consistent with the hypothesis that the fluctuations arise from a single population of channels whose duration is exponentially distributed. The average channel duration, τ, compared on the same four neurones was (mean±s.d.) 26.7 ± 7.7 ms (71 observations) and 4.8 ± 1.3 ms (50 observations) for GABA- and glycine-activated channels, respectively. Estimates of durations derived from amino acid responses obtained on different neurones were 20 ± 6.6 ms for GABA- (271 observations) and 5.2 ± 1.6 ms for glycine-activated channels (71 observations).

From these results it is clear that single channel properties activated by GABA and glycine are not identical, with GABA-induced events being lower in conductance and longer in duration than those induced by glycine. The differences in the properties of the single channel events may reflect differential binding of the amino acids to the same receptor–channel complex, as has been suggested to account for different single channel events activated by various cholinergic agonists applied to endplate regions of muscle membranes[6]. However, a single receptor site for neutral amino acids on spinal neurones is not supported by several lines of investigation. Convulsant drugs can selectively antagonise the pharmacological responses of spinal and sensory neurones to either of the amino acids without affecting responses to the other[7–10]. The amino acids do not affect each other's affinity for receptor sites as reflected in binding studies on membranes extracted from the central nervous system (refs 11–13 and E. J Peck, personal communication). Structure–activity studies with conformationally restricted GABA analogues have indicated an 'active conformation' of GABA which cannot be matched by any configuration of glycine[14]. Some membranes respond to GABA but not to glycine[15,16]. We have also found a selective antagonism of the amino acid responses on cultured spinal neurones by specific convulsants (refs 17, 18 and unpublished observations) and a selective enhancement of GABA responses by barbiturates[19,20] and benzodiazepines[21]. Cultured cells grown in the presence of GABA respond to glycine but not GABA, while cells grown in glycine respond to GABA, but not glycine[22]. These findings, coupled with the present results, suggest that the different single channel properties activated by GABA and glycine reflect engagement of physically distinct receptor–channel complexes on neuronal membranes.

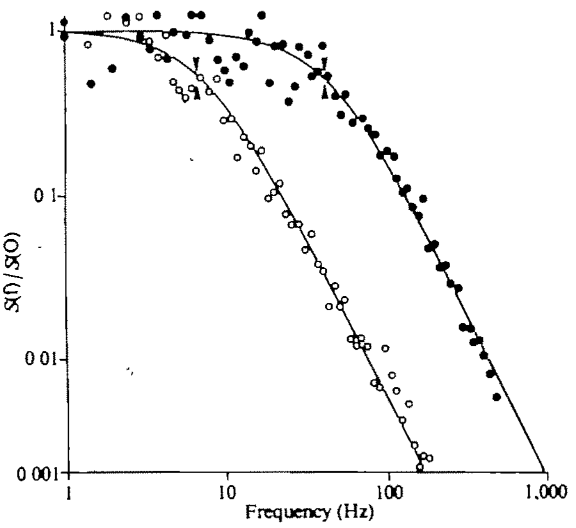

Fig. 2 Power density spectra of amino acid-induced current fluctuations show different durations for GABA- (○) and glycine- (●) activated channels. These spectra were calculated from results obtained on the same spinal neurone as in Fig. 1. The spectra shown are the differences between spectra derived from amino acid-induced fluctuations and those derived from baseline fluctuations. They have been normalised by dividing each spectral density point, $S(f)$, by the zero-frequency asymptote of the spectrum, $S(0)$. Points across frequency bands have been averaged to smooth the spectra. The points are approximated by lorentzians (solid lines) of the form $S(f)/S(0)=1/[1+(f/f_c)^2]$. Double arrowheads indicate the half-power frequency, f_c which is related to mean channel duration, τ, by $\tau=\frac{1}{2}\pi f_c$. In this cell, f_c for GABA is 6.5 Hz and f_c for glycine is 41 Hz, corresponding to channel durations of 24.5 and 3.9 ms, respectively.

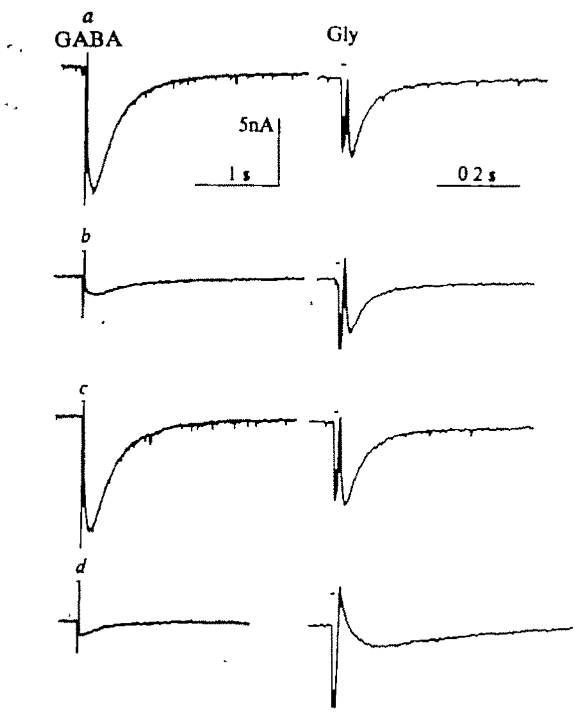

Fig. 3 GABA and glycine interact on cultured spinal neurones The cell illustrated was voltage clamped to −80 mV and GABA and glycine iontophoresed either separately (*a*, control; *c*, recovery) or together (*b*, during prolonged iontophoresis of GABA; *d*, during prolonged iontophoresis of glycine). The amplitude of the GABA current response is markedly depressed and its time course slowed when superimposed on a prolonged, desensitising dose of GABA (*b*, left-hand trace). A depression of the glycine current response without apparent change in time course is also evident during prolonged GABA iontophoresis (*b*, right-hand trace). Conversely, the GABA response is markedly depressed without apparent change in time course during prolonged glycine iontophoresis (*d*, left-hand trace). The glycine response is also considerably depressed and its time course slowed during the prolonged, desensitising glycine application (*d*, right-hand trace).

This notion is not supported by observations made on amino acid current responses elicited on membranes acutely desensitised to one or the other amino acid. For example, sustained applications of GABA induced a current and conductance response which rapidly desensitised. Current and conductance responses to glycine iontophoresed onto membranes desensitised to GABA were depressed in a reversible manner relative to those obtained in the absence of GABA, while the time course of the responses did not seem to be altered (Fig. 3*a–c*). (The time course of desensitised GABA and glycine responses was always slower than those obtained in control.) Conversely, sustained application of glycine sufficient to induce desensitisation of glycine responses reversibly depressed the current and conductance response to GABA relative to control with little change in its time course (Fig. 3*d*). Similar depressions of one amino acid response during sustained iontophoresis of the other were observed in each of 11 spinal cord cells where the interaction was examined. The depressions of the amino acid responses were not associated with a change in the driving force for the amino acid responses (unpublished observations).

One explanation for these results is that both amino acids can compete for each receptor site but only activate conductance at the appropriate receptor. The inappropriate amino acid would thus interfere with channel activation by the appropriate amino acid at the receptor. However, a variety of studies have failed to show an interaction between the amino acids (refs 11–13, 22, E. J. Peck, personal communication) and we have found that GABA responses elicited on cultured dorsal root ganglion cells are not altered by sustained application of glycine, which does not affect the membrane properties of the cells (unpublished observations). Thus, it seems unlikely that the interaction takes place at the receptor level. Alternatively, GABA and glycine receptors may share a common channel which can still be activated by glycine when GABA receptors are largely desensitised but which is difficult to activate by GABA when glycine receptors are only partially desensitised and thus almost continuously using these channels. This latter explanation suggests that postsynaptic Cl^- conductance mechanisms might be coupled to several amino acid receptors and that the properties of the resulting single channel event are determined by the type of amino acid released from presynaptic terminals.

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Acetylcholine receptors in organ-cultured human muscle fibres

ORGAN culture of nerve and muscle facilitates investigations which cannot easily be carried out *in situ*[1,2]. We have developed a method for maintaining human muscle in organ culture which should allow us to investigate normal as well as various pathological properties of muscle fibres. We report here the use of these preparations to compare junctional and extrajunctional channels, induced by acetylcholine (ACh), in human muscle. In addition we have made a preliminary study of miniature endplate currents, in normal and myasthenia gravis endplates, following organ culture.

Human intercostal nerve-muscle obtained from myasthenia gravis and control patients was dissected in sterile conditions to give small bundles of fibres (~100). Fibre bundles were pinned through their tendons and mounted, under slight tension, on Sylgard frames. They were maintained in Trowell's T-8 culture medium (Gibco) or Dulbecco's minimal essential medium (Gibco) containing 100 IU ml^{-1} penicillin and streptomycin and 0.02 mM glutamine in an atmosphere of 95% O_2/5% CO_2 (ref. 3). Cultured muscle fibres had 'overshooting' action potentials and resting potentials of over 70 mV, and remained viable for periods of at least 2 weeks at a constant temperature of 23 °C. At this temperature the rate of metabolism is greatly reduced[4]. Electrophysiological methods were as previously described[5].

Extrajunctional ACh sensitivity was studied at the muscle–tendon junction, where it is known to occur in other species[6,7]. A voltage recording intracellular microelectrode was inserted near the tendon to measure the response to a 1–2 nC ionophoretic pulse of ACh applied to the surface of the fibre. The ACh pipette was moved in steps of 50–100 μm (Fig. 1). Some fibres (approximately 5–50%) of normal human muscle were found to have a marked ACh sensitivity at the tendon junction. The sensitive area covered a length of ~800 μm, that is, a small fraction of the total fibre length of approximately 1–2 cm. The peak of sensitivity sometimes occurred several hundred μm away from the

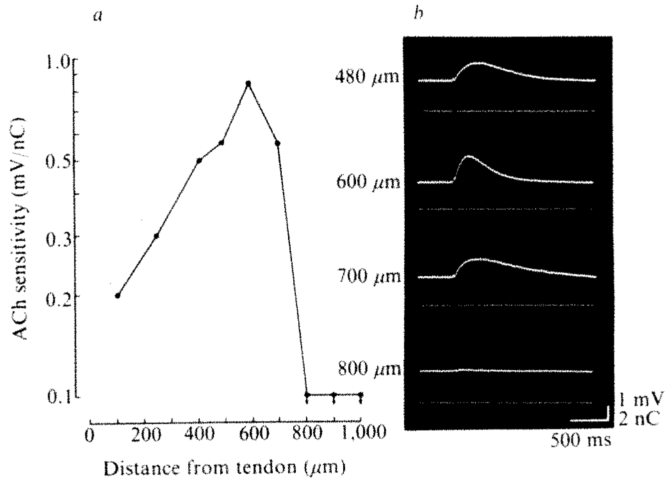

Fig. 1 *a*, Distribution of extrajunctional acetylcholine sensitivity, at the muscle–tendon junction of a fresh normal human muscle fibre, measured in response to 2 nC pulses of ACh applied from a micropipette at various distances from the tendon. Note that the peak of sensitivity in this fibre apparently occurs at 600 μm from the tendon although the presence of connective tissue in the tendon region makes the actual site of peak sensitivity uncertain. At 800 μm or more from the tendon, sensitivity was undetectable. *b*, Examples of the potential changes produced by ACh applied to the tendon region (at 480, 600, 700 and 800 μm from the tendon); same experiment, as shown in graph. The drug ejection currents passing through the micropipette are monitored on the lower trace in each pair. Calibration 1 mV or 2 nC, 500 ms.

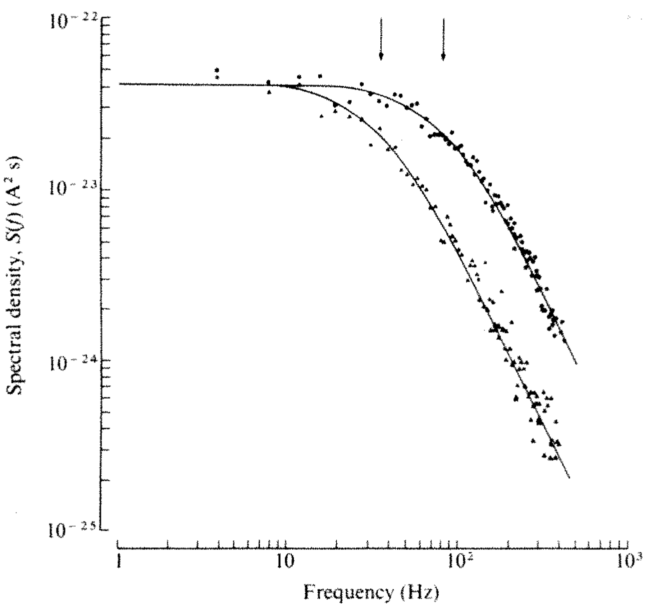

Fig. 2 Power spectra of current noise produced by steady ionophoretic application of acetylcholine to a voltage clamped endplate (●) and an extrajunctional site at the muscle–tendon junction (▲) in human muscle cultured at 23 °C for 5 d. The spectra are from two fibres, in the same bundle, at clamp holding potential $V_m = -80$ mV, temperature, 21 °C. Endplate spectrum (●) was obtained from 136 averaged spectra (four noise runs). Half-power frequency indicated by arrow is $f_c = 87$ Hz, hence $\tau_{noise} = 1.8$ ms. Mean current, $\mu_I = 19.7$ nA and the single channel conductance calculated from the fitted lorentzian curve is, $\gamma = 25.14$ pS. Extrajunctional spectrum (▲) was obtained from 76 averaged spectra (three noise runs). Half-power frequency indicated by arrow is $f_c = 35$ Hz, hence $\tau_{noise} = 4.5$ ms. Mean current, $\mu_I = 4.8$ nA and the single channel conductance calculated from the fitted curve is, $\gamma = 11.75$ pS. Vertical axis applies to the extrajunctional spectrum; multiplication of the ordinate values by 3.03 gives the spectral density values (A^2 s) for the endplate noise. After feeding into a 1,000-Hz active ($1/f^8$) low pass filter, data was digitalized at 2,000-Hz sample rate and edited to remove spontaneous miniatures and artefacts. Control noise, taken immediately before application of ACh, has been subtracted. Fast Fourier transform was calculated for 512 point segments. The single channel conductance was normally estimated from both the variance, σ^2, of the current noise and the zero frequency asymptote, $S(0)$, of the spectrum; $\gamma = \sigma^2/\mu_I(V_m - V_{eq})$ and $\gamma = S(0)f_c/\mu_I(V_m - V_{eq})$ where the equilibrium potential, V_{eq}, for ACh was taken as zero mV.

tendon (Fig. 1). Normally, sensitivity was not detectable between the tendon junction and the endplate. Fibres cultured for up to 14 d did not acquire sensitivity in this region. Also, in only one out of eight cultures kept at 36 °C for up to 14 d did we find evidence for denervation supersensitivity. This is in sharp contrast to what has been found in rat muscles which rapidly acquire supersensitivity in the whole fibre after denervation in similar culture conditions[2].

Acetylcholine sensitivity was not detected in the muscle tendon region of any of the myasthenic muscles studied (16 fibres, 3 muscles). This is probably related to the same process which causes reduced ACh sensitivity of endplates in myasthenic fibres.

Properties of junctional and extrajunctional channels were compared in normal human muscle cultured at 23 °C by analysing the voltage clamped noise[8-10] which occurred during steady ionophoretic application of ACh to a junctional site or to a tendon region. Power density spectra of current fluctuations are shown in Fig. 2. In most cases spectra were fitted with a single lorentzian component (1/frequency^2), and the frequency, f_c, at which the power is reduced to one half of its maximum value, gave the mean life-time of the open channel, τ_{noise}, according to $\tau_{noise} = 1/(2\pi f_c)$. Power spectra of junctional and extrajunctional noise are similar in form (Fig. 2) but their half-power frequencies differ revealing that the open-state of extrajunctional channels is prolonged when compared with that of endplate channels.

In the example shown in Fig. 2 extrajunctional channels have a mean life-time of 4.5 ms compared with 1.8 ms for the junctional channel from the same muscle. The mean open-time of junctional channels in culture was τ_{noise} (junctional) = 1.55 ± 0.08 ms ($n = 7$ fibres); the comparable value for extrajunctional channels was τ_{noise} (extrajunctional) = 3.52 ± 0.27 ms ($n = 5$ fibres), $V_m = -80$ mV, temperature, 21 °C. The single channel conductance, γ, derived from both the variance of the current noise and the zero frequency asymptote of the spectra, was in the range 20–25 pS for endplate channels and 11–16 pS for extrajunctional channels. The values of these channel parameters are similar to those obtained in the same muscles before culturing (see also ref. 11).

In both normal and myasthenic endplates miniature endplate currents (m.e.p.cs) are present for 5–7 d in muscles cultured at 23 °C indicating the time required for denervation of the endplates. It was therefore possible to assess the sensitivity of fresh and cultured endplates by comparing their m.e.p.c. amplitudes. Myasthenic m.e.p.cs are small apparently due to binding of

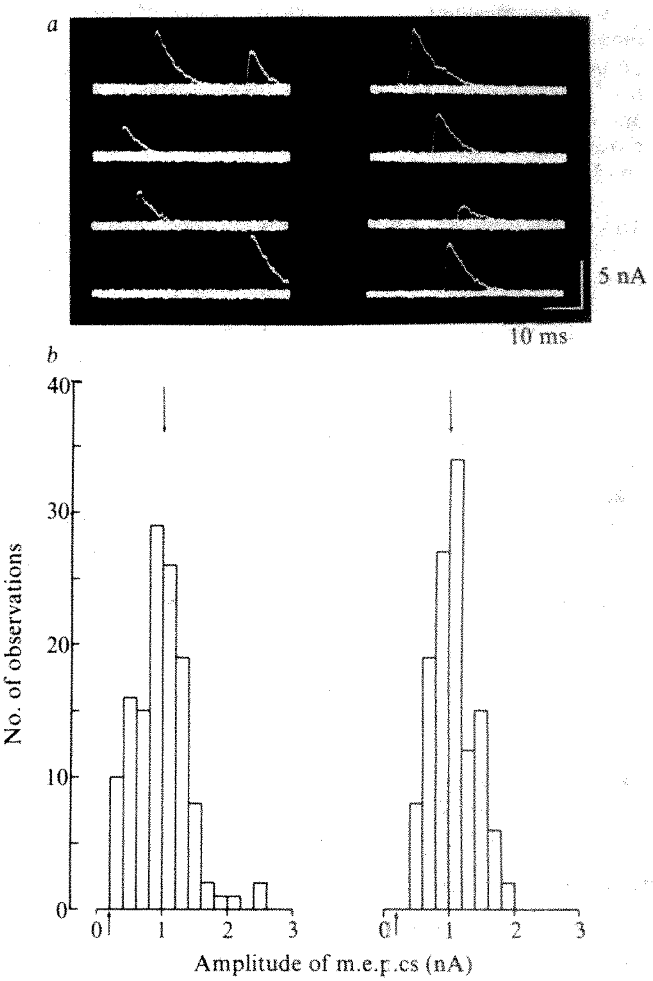

Fig. 3 *a*, Examples of miniature endplate currents at a fresh normal human endplate (left) and at an endplate from the same muscle after 5 d in culture (right). m.e.p.cs were recorded under voltage clamp ($V_m = -80$ mV, temperature, 21 °C) in the presence of prostigmine 2×10^{-7} g ml^{-1} through a 500-Hz low pass filter which prolongs the rising phase and slightly attenuates the amplitude. Calibration, 5 nA and 10 ms. *b*, Amplitude distributions of m.e.p.cs at a voltage clamped myasthenic human endplate in fresh muscle (left) and at an endplate from the same muscle after 6 d in culture (right). Mean m.e.p.c. amplitude (arrows) at the fresh endplate = 0.99 ± 0.06 nA (mean ± s.e.m.); mean m.e.p.c. amplitude at an endplate after 6 d culture = 1.06 ± 0.03 nA. m.e.p.cs were obtained in the presence of prostigmine 2×10^{-7} g ml^{-1} ($V_m = -80$ mV, temperature, 21 °C). Background noise level is indicated by arrows on abscissa.

anti-receptor antibodies to the ACh-receptor[12], which may cause a loss of functional receptors[13,14], and also a reduction in their number through an increased rate of degradation[15-17]. As circulating anti-receptor antibody occurs in the plasma of myasthenic patients[18], the possibility arises that in its absence the m.e.p.cs would increase in amplitude if antibody could detach from receptors or if new receptors were incorporated into the membrane; although the latter process should be far from optimal in culture conditions at 23 °C.

The amplitude of m.e.p.cs at normal human endplates is not markedly changed after 5–6 d in our culture conditions. Figure 3*a* shows examples of m.e.p.cs recorded from a voltage-clamped normal endplate at zero culture time and from another endplate in the same muscle 5 d later ($V_m = -80$ mV, temperature, 21 °C, prostigmine 2×10^{-7} g ml^{-1}). In cultures of three myasthenic muscles there was no marked change in m.e.p.c. amplitudes after 5–7 d. Amplitude distributions of myasthenic m.e.p.cs from one endplate before culture and from another endplate in the same muscle 6 d later are shown in Fig. 3*b*.

We conclude that extrajunctional ACh receptors are normally present in some adult human muscle fibres and that the channels they operate differ from endplate channels in their kinetic properties. This is analogous to the situation in denervated frog[8,19], rat and mouse[20] muscle where properties of junctional and extrajunctional channels show differences comparable to those described here. However, the single channel life-time and conductance of the extrajunctional channels in adult human muscle are smaller than those in cultured human myotubes[21]. This dissimilarity as well as that between junctional and extrajunctional receptors in adult fibres may reflect differences in the receptor molecules themselves or in their membrane environment.

Finally our experiments indicate that if some of the reduced ACh sensitivity of myasthenia gravis endplates results from binding of an anti-receptor antibody, as has been suggested, then the antibody–receptor combination is practically irreversible when the muscle is maintained at 23 °C or the antibodies may unbind but leave the receptors still in a non-functional state.

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Is the red cell calcium pump regulated by ATP?

THE very low physiological level of calcium in the human red cell is maintained by a powerful ATP-fuelled Ca extrusion pump which has been extensively studied[1]. Recently evidence has been accumulating that the kinetic properties of the active Ca flux and associated (Ca+Mg)ATPase activity depend very much on the experimental conditions and/or the manner of preparation of the red cell membranes or resealed ghosts[2-4]. The variability may reflect the nature or degree of interaction between the membrane Ca pump and a cytoplasmic activator protein described recently[5-7], which could be involved in regulation of physiological Ca levels. In previous work with whole red cells[8,9] or resealed ghosts[10] the concentrations of the major Ca pump ligands, ATP and/or Ca, have been changing during the course of the reaction. Because of the possible importance of the ligand conditions in assessing the physiological functioning of the Ca pump, we have looked at (Ca+Mg)-dependent ATP hydrolysis in resealed ghosts with buffered ATP and Ca levels and at the active Ca flux with a buffered ATP level. The experiments reported here show that both the ATP hydrolysis and the Ca flux are activated with two distinct ATP affinities and this raises the possibility of regulation of the Ca pump by ATP in the region of the lower affinity.

For the ATPase measurements, the internal ATP concentration was clamped at a fixed level by incorporating [γ-^{32}P]ATP and a regenerating system into the ghosts. The regenerating system consisted of creatine kinase and creatine phosphate in chemical and isotopic equilibrium with the ATP. The method (details of which are given in the legend to Fig. 1) was developed previously[11] and has the advantage of allowing measurement of ATP hydrolysis at very low concentrations. The free Ca levels in the cells were maintained in the range 10^{-8} to 10^{-4} M by incorporating Ca buffers into the cells and equilibrating the Ca concentrations across the membranes by means of the Ca-ionophore A23817. Figure 1 shows a time course of ^{32}Pi release in cells prepared to contain 50 μM ATP and about 6 μM free Ca^{2+}. Measurements of the internal Ca by flame photometry showed that it was approximately equal to the external Ca concentration at the beginning of the test incubation and remained constant (not shown). The rate of ^{32}Pi release was constant for about 15 min during which time the total ATP content of the ghosts must have turned over about 40 times. Linearity has been observed regularly at 1–1,000 μM ATP demonstrating effective regeneration of ATP even at the high rates of ATP hydrolysis observed.

In several experiments in which ghosts were prepared to contain different Ca-buffer mixtures, and either 10 or 500 μM ATP, the free Ca^{2+} concentration which sustained the half-maximal ATPase activity was about 0.2 μM, that is, a 'high affinity' (see refs 3, 4).

Figure 2 shows the rate of (Ca+Mg)-dependent ATP hydrolysis as a function of the total ATP concentration in cells, with an optimal but relatively low free Ca^{2+} concentration of 6.3 μM. The curve is clearly biphasic. The kinetic parameters calculated from the curve show a high affinity component with K_m 1–2 μM and a V_{max} of about 4.7 mmol per l cells per h, and the low affinity region has a K_m of about 180 μM with an overall maximal rate of about 16 mmol per l cells per h. The rate of ATP hydrolysis in resealed ghosts without Ca, that is Mg-dependent ATPase, was subtracted from the total activity with Ca and Mg at all ATP concentrations. The rate of Mg-ATPase did not vary between 10 and 500 μM ATP and the measured value of 0.8 mmol per l cells per h was used to make the correction. The rate of (Ca+Mg)ATPase is presumably somewhat underestimated at very low ATP concentrations (<10 μM) but this does not alter the activation pattern.

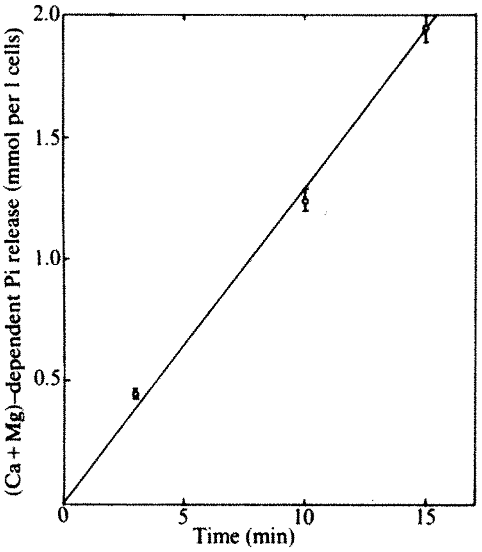

Fig. 1 (Ca + Mg)-dependent ATP hydrolysis in resealed red cells containing buffered concentrations of Ca^{2+} ions and ATP. See ref. 11 for details. Preparation of [γ-^{32}P]ATP and ^{32}P-creatine phosphate in chemical and isotopic equilibrium: 250 μM of ATP(containing [γ-^{32}P]ATP 1×10^6 c.p.m.) was added to 1 ml of a solution containing 50 mM creatine phosphate, 0.5 mM creatine, 12.5 mM $MgCl_2$, 25 mM inosine, 0.5 mM ouabain, 50 mM HEPES (Tris) (*p*H 7.4 at 22 °C) and 25 units of creatine kinase and hexokinase. After 1 h incubation at 37 °C, 5 μmol of HEDTA (*p*H 7.4 at 22 °C) was added, the solution was diluted 5 times and kept at 0 °C. Preparation of resealed ghosts and measurement of ATP hydrolysis: red blood cells were washed 3 times with a standard 'wash solution' which contained: 130 mM NaCl, 10 mM KCl, 2.5 mM $MgCl_2$ and 10 mM HEPES (Tris) (*p*H 7.4 at 22 °C). The cellular ATP was depleted by incubating the cells for 60 min at 37 °C in a medium containing 6 mM inosine and 6 mM iodoacetamide. The cells were then washed 3 times at room temperature to remove inosine and iodoacetamide and packed at 1,500*g* for 10 min. Packed cells were lysed in 9 times their own volume of the diluted ice-cold solution containing the ATP regeneration system. After 2 min 2 M NaCl was added to restore the tonicity to 310 ideal milliosmolar, and the ghosts were incubated at 37 °C for 10 min. The resealed ghosts were then washed 3 times, the first and third time with ice-cold medium and the second wash at room temperature. The final intracellular concentrations were: NaCl, 130 mM; $MgCl_2$, 2.5 mM; HEDTA, 1 mM; ATP (+[γ-^{32}P]ATP), 50 μM; creatine phosphate, 10 mM; HEPES (Tris), 10 mM *p*H 7.4; creatine, 100 μM; ouabain 0.1 mM; creatine kinase 5 units per ml and hexokinase 5 units per ml. The ghosts were packed at 25,000*g* for 1 min, suspended to 3–5% haematocrit in ice-cold wash solution containing 0.1 mM ouabain, and $CaCl_2$ 0.71 mM plus HEDTA 1 mM, calculated to give 6.3 μm free Ca^{2+} (see ref. 12). Ca ionophore (A23187) was then added to a final concentration of 10 μM and the ghosts were incubated for 20 min at 0 °C and for 5 min at 37 °C before the measurements were begun. At various times at 37 °C the ATP hydrolysis was terminated by the addition of glucose to a final concentration of 15 mM and further 10 min incubation at 37 °C (this converts the remaining creatine phosphate and ATP to glucose-6-phosphate). The membranes were precipitated by 5% perchloric acid. 1 ml of membrane free extract was shaken for 30 s with 4 ml 0.5% ammonium molybdate in 5% (w/v) perchloric acid and 2 ml isobutanol, and the tubes were centrifuged at 1,000*g* for 30 s. Measured portions of the isobutanol layer were transferred to vials containing 0.5 ml of 2 M Tris solution and 8.5 ml Bray's scintillation fluid, and counted. The total ^{32}P counts were estimated by boiling 1 ml of the ghost suspension with 1 M HCl for 15 min and extracting ^{32}P as above. The rate of ATP hydrolysis is calculated from the fraction of breakdown of total phosphate substrate (creatine phosphate + ATP) per given time. Each point represents the average value ± 1 s.e.m. from tubes incubated in triplicate.

Experience showed that to observe biphasic curves the free Ca^{2+} within the cell must be less than about 1 mM. At higher concentrations (3–6 mM) we have observed an apparent hyperbolic saturation by ATP with a K_m of 5–10 μM and a severely depressed maximal rate of 3–6 mmol per l cells per h. The difference in the observed type of behaviour at low and high Ca is attributable to this inhibitory effect of high Ca concentrations, which is much more effective at the higher ATP concentrations than in the low ATP concentration range (not shown).

The curve in Fig. 2 could indicate that ATP combines twice with the Ca pump per turnover but it might also be explained by the action of two different (Ca + Mg)-dependent enzymes with only one involved in Ca transport. It was therefore also important to look at the ATP dependence of the active Ca flux. For the flux experiment of Fig. 3 the ATP levels were buffered again by the use of the creatine phosphate–creatine kinase system and the cells were loaded with 2.5 mM Ca(+ ^{45}Ca). Here, of course it is not possible to use the ionophore and so the internal Ca concentration drops during the course of the incubation. The cells in this experiment contained less than 1 mM total Ca immediately before the test incubation. The rate of ^{45}Ca efflux was constant for 5–15 min at the different ATP concentrations. A biphasic behaviour is clearly observed. The kinetic parameters for the two regions are respectively $K_m \approx$ 2 μM, V_{max}, 2.2 mmol per l cells per h and $K_m \approx 190$ μM with an overall V_{max} of 7.3 mmol per l cells per h. Again if much higher initial Ca concentrations were used the biphasic pattern could not be clearly distinguished. In Fig. 3 the rates of Ca efflux are appreciably lower than the ATPase measured with low free Ca^{2+} concentration plus ionophore (see Fig. 2). This is due to a significant inhibitory effect of Ca even at the 0.5–1 mM level present in the flux experiment. The Ca inhibition seems to affect the V_{max}, or turnover rate of the Ca pump without altering the apparent affinities or K_m for ATP. In parallel measurements of ATP hydrolysis estimated as in Fig. 2 but without ionophore, and Ca flux, made with ghosts containing 0.5–1 mM Ca^{2+} and 10 μM or 500 μM ATP, we have observed a ratio of Ca efflux to (Ca + Mg)-dependent ATP hydrolysis of 0.93 and 1.08 at the two ATP concentrations. This confirms the figure reported originally by Schatzmann[10].

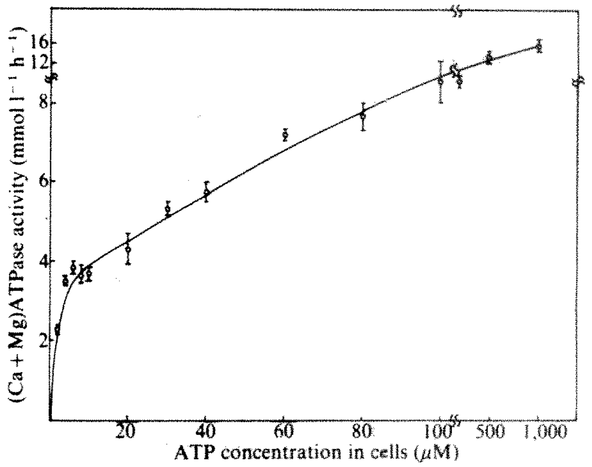

Fig. 2 ATP dependence of the (Ca + Mg)ATPase in resealed ghosts. Ghosts were prepared essentially as described in the legend to Fig. 1 except that: (1) the cells had been starved for 24 h at 37 °C in the glucose free wash solution containing also 0.2 mM EGTA with no added Ca, and chloramphenicol (25 μg ml^{-1}), before a 2-h incubation with inosine and iodoacetamide; and (2) the lysing solution contained the appropriate concentrations of [γ-^{32}P]ATP, together with the ATP regeneration system. For measurement of (Ca + Mg)ATP hydrolysis the cells were incubated at 37 °C for 15–60 min according to their ATP concentration. The rate of Mg-dependent ATP hydrolysis was measured over a 120 min incubation period in cells containing 10 μM or 500 μM ATP, and 1 mM EGTA.

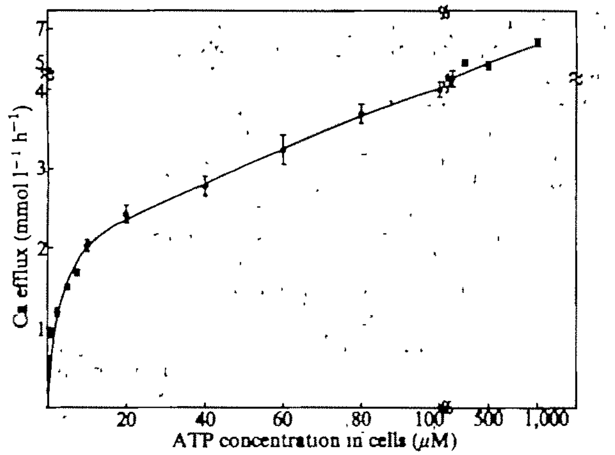

Fig. 3 ATP dependence of active Ca efflux from resealed ghosts. Red cells were washed as in the legend to Fig. 1 and starved as described in the legend to Fig. 2. 0.5 ml of packed starved red cells was lysed in 4.5 ml of ice-cold lysing solution containing: ATP, 1–1,000 μM as indicated, creatine phosphate, 10 mM creatine kinase 25 units. $CaCl_2$ (containing ^{45}Ca 2×10^6 c.p.m.) 2.5 mM; HEDTA, 1 mM; $MgCl_2$, 2.5 mM; HEPES (Tris), 10 mM(pH 7.4 at 22 °C); inosine, 5 mM; and ouabain 0.1 mM. After 2 min 2 M NaCl was added to restore tonicity to 310 ideal milliosmolar. The cells were incubated for 10 min at 37 °C and washed as in the legend to Fig. 1 (EGTA, 0.5 mM was added to the warm wash solution). The cells were then suspended at 2–3% haematocrit in ice-cold wash solution. The Ca flux was initiated by transferring the tubes to a 37 °C water bath. At various times the efflux was terminated by removal of 0.5 ml of the suspension into 0.7 ml of ice-cold wash solution containing 170 μM $LaCl_3$. The cells were spun down for 2 min at 12,000g in an Eppendorf Microcentrifuge, and 1 ml supernatant was transferred into vials containing 9 ml of Brays scintillation fluid, and counted. Radioactivity in the supernatant at zero time was subtracted from that at all other incubation times. The total ^{45}Ca counts present initially in the cells was obtained by allowing complete efflux in a 2-h incubation at 37°C. The concentration of Ca in the cells before the 37°C incubation was estimated either from the total radioactivity per known volume of cells and the specific activity of the ^{45}Ca in the lysing solution, or by flame photometry. For the latter measurement 50 μl of ghosts of known haemotocrit (25–50%) were lysed in 1 ml of perchloric acid 1.5% w/v. The precipitated protein was spun down and the supernatant Ca was read against standard $CaCl_2$ solutions, 25–100 μM made up in 1.5% PCA and the appropriate volume of wash solution (containing NaCl) added with the cell suspension Both methods give similar results. In the experiment of Fig. 3 the initial Ca concentrations were 0.8–1.1 mM. The rate is calculated from the fractional efflux of the total Ca per given time. Each point represents the average value ±1 s.e.m. from tubes incubated in triplicate.

The biphasic ATP activation curve and the constancy of the ratio of Ca flux to ATP hydrolysis at 10 and 500 μM ATP suggest strongly that the ATP hydrolysis at both high and low ATP levels (Fig. 2) is involved in the working of the Ca pump.

In previous observations of ATP activation of the ATPase, using broken membranes somewhat conflicting results have been reported. Wolf[13] and Schatzmann[14] observed an apparent simple saturation behaviour with K_m values for Mg-ATP in the range 20–50 μM. Wolf *et al.*[15], working with a partially purified (Ca+Mg)ATPase, and Sharff and Foder[16], have reported Hill coefficients of <1 for activation by ATP but no evidence for a very high affinity activation as in the present resealed ghost experiments. On the other hand Sarkadi *et al.*[17], looking at the active Ca flux in inside-out vesicles have seen a high ATP affinity. It seems possible that the differences between the resealed ghosts and broken membranes reflect changes that occur during the membrane preparations or result from the inhibitory effects of high Ca^{2+} mentioned above.

The reaction mechanism of the Ca pump-associated (Ca+Mg)ATPase involves a phosphorylated intermediate[18]. Rega and Garrahan[19] have shown that formation of the phosphoenzyme requires ATP at 1–2 μM, that is, in the high affinity range. Thus it seems possible that the increase in the turnover rate at the higher ATP concentrations is a regulatory function of the nucleotide acting in a non-phosphorylating role. The cell membrane (Na+K)ATPase is phosphorylated by ATP acting at a high affinity site and in addition an extra non-phosphorylating function of the ATP at a low affinity site is well documented for both kidney membranes[20] and resealed red cells where we previously reported biphasic ATP activation curves[11]. The non-phosphorylating function of ATP involves regulation of the rate of a conformational transition, which is probably involved in the transport of K^+ ions[20–22].

As the membrane Ca pump and Na pump show a number of similarities in the reaction mechanism it would be interesting if regulation of transport linked conformational changes by ATP is found to be another common feature of the active cation pumps.

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Physiological correlation between lactate dehydrogenase genotype and haemoglobin function in killifish

ERYTHROCYTE organic phosphate concentration is known to be an heritable trait in both humans and rats[1–3]. In humans this trait has been correlated with genetic variability of both pyruvate kinase and hexokinase[2]. However, similar phenomena have not been reported in lower vertebrates. During our studies on the allosteric regulation of fish haemoglobins by ATP (ref. 4) we observed significant variability in red cell ATP concentrations among individuals of the killifish *Fundulus heteroclitus*, and wondered whether this variability in organic phosphate could be the result of a genetic component. Consequently, we analysed *F. heteroclitus* for red cell ATP, concomitantly screening for enzyme variants. We report here that not only are ATP levels under genetic control, but they are also highly correlated with lactate dehydrogenase (LDH) phenotype. To our knowledge, this is the first such correlation reported. Moreover, as ATP is the major allosteric modifier of *F. heteroclitus* haemoglobin–oxygen affinity[5,6], this study provides a basis for an association

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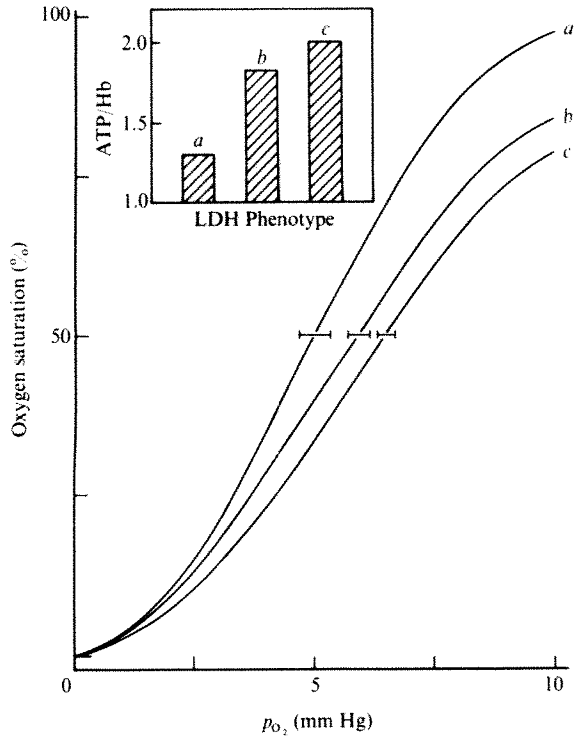

Fig. 1 Oxygen equilibria curves for whole blood from *F. heteroclitus* determined with an Aminco oxygen dissociation analyser. The ordinate is the fractional saturation of haemoglobin by oxygen and the abscissa the partial pressure of oxygen (p_{O_2}) in mm Hg. The standard errors of the mean p_{O_2} ($n = 6$) for 50% saturation are represented by horizontal bars. Intra-erythrocyte ATP/Hb ratios for the three phenotypes are presented in the inset. ATP was determined by the firefly luciferase assay[11]. *Fundulus* haemoglobin concentrations were determined as described previously[5]. The values for ATP/Hb are: *a*, $LDH\text{-}B^aB^a = 1.27 \pm 0.08$ ($n = 6$); *b*, $LDH\text{-}B^aB^b = 1.83 \pm 0.11$ ($n = 5$); *c*, $LDH\text{-}B^bB^b = 2.00 \pm 0.06$ ($n = 5$). All animals were acclimated to laboratory conditions for 30 d before assays. Temperature, 20.5°C, *p*H 7.4.

between LDH and the fish's external environment through the physiological role of haemoglobin at the organism–environment interface.

There are three major electrophoretically distinguishable phenotypes of the heart-type LDH (the *LDH-B* locus) in *F. heteroclitus*[7,8]. The polymorphism is due to two co-dominant alleles which exhibit a dramatic north–south cline in gene frequency along the Atlantic coast of the US[9,10]. The two common *LDH-B* homozygous phenotypes yield fast ($LDH\text{-}B^aB^a$) and slow ($LDH\text{-}B^bB^b$) bands on electrophoresis, whereas the heterozygote phenotype ($LDH\text{-}B^aB^b$) gives the classic five-banded pattern[8]. The *B* locus is the only LDH expressed in the red blood cells of this fish[8]. When intra-erythrocyte ATP levels (expressed as molar ratios of ATP/Hb) are compared for individuals of different *LDH-B* phenotype, a significant association ($F_{[2,13]} = 20.58$, $P < 0.001$; index of association $\omega^2 = 0.76$) is found (see inset in Fig. 1).

ATP is the major organic phosphate in the red blood cells of *F. heteroclitus*[5]. Its role as an allosteric modifier of fish haemoglobins is similar to that of 2,3-diphosphoglycerate in mammalian red blood cells, in that it decreases the affinity of haemoglobin for oxygen[5]. A decrease in haemoglobin–oxygen affinity is characterised by an increase in the partial pressure of oxygen at which one-half of the haemoglobin is saturated with oxygen (the P_{50}). As the intra-erythrocyte ATP/Hb ratio is different for each of the *LDH-B* phenotypes, we expected to find differences in haemoglobin–oxygen affinity also. In agreement with this expectation (Fig. 1), $LDH\text{-}B^aB^a$ homozygotes with the lowest ATP/Hb ratio have the highest oxygen affinity and $LDH\text{-}B^bB^b$ homozygotes with the highest ATP/Hb value have the lowest oxygen affinity. At the same partial pressure ($p_{O_2} = 10$ mm Hg) the haemoglobin from $LDH\text{-}B^aB^a$ fish is 96% saturated with oxygen, whereas that of $LDH\text{-}B^bB^b$ individuals is only 80% saturated. Such differences in oxygen loading would be exaggerated *in vivo* when blood *p*H is reduced following an increase in temperature and/or anaerobic metabolism[5].

Our work on the haemoglobin components of *F. heteroclitus* indicates that the shift in oxygen equilibria does not result from a difference in the distribution of the haemoglobin components between the *LDH-B* phenotypes, as all individuals express the same complement of components. In addition, each isohaemoglobin responds similarly to the changes in *p*H, temperature and ATP concentration[5]. Therefore, the differences in intra-erythrocyte ATP concentrations reported here must be either the result of: (1) functional differences between the LDH-B allozymes which affect cellular ATP levels, or (2) the regulation of ATP levels by other enzymes which are genetically linked to the *LDH-B* locus. Studies are now underway to answer this question. Also, selection experiments are in progress to determine the feasibility of oxygen and *p*H as selective agents for the *LDH-B* phenotypes of *F. heteroclitus*.

Whatever the molecular basis for these ATP differences, it is clear that the $LDH\text{-}B^aB^a$ phenotype has a lower intra-erythrocyte ATP concentration than $LDH\text{-}B^bB^b$ individuals and as a consequence, a higher haemoglobin–oxygen affinity (Fig. 1). Fish with the $LDH\text{-}B^aB^a$ phenotype might therefore have a selective advantage when competing with individuals of the other two phenotypes in a low oxygen environment. Final resolution of this phenomenon may help resolve the controversy between exponents of the 'selectionist' and 'neutralist' evolutionary theories concerning the maintenance of protein polymorphisms in natural populations.

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Immunoprecipitation of protein kinase activity from adenovirus 5-infected cells using antiserum directed against tumour antigens

THE function of transformation proteins coded for by RNA- and DNA-containing tumour viruses is a major question in oncology. A large number of diverse alterations are produced in cells following viral transformation, and such changes are thought to be the result of the action of one or a very few viral gene products. Until recently, very little was known concerning the mechanism of action of such transforming proteins.

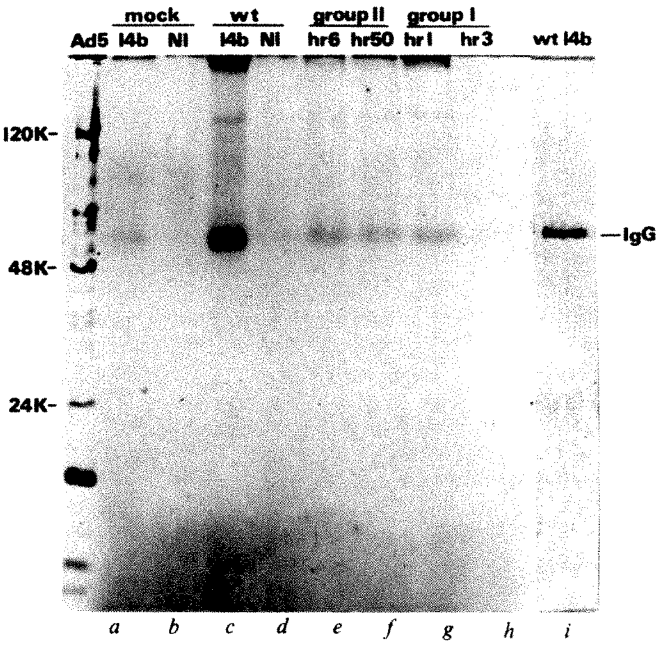

Fig. 1 *In vitro* phosphorylation of immunoprecipitates from Ad 5- and mock-infected cells. Proliferating KB cells were infected with Ad 5 (50 plaque-forming units per cell) or were mock infected. At 9 h post-infection, cytoplasmic extracts were prepared and these were immunoprecipitated using a method similar to that of Schaffhausen *et al.*[9]. Cells were scraped off 100-mm Petri dishes, washed twice with cold phosphate-buffered saline, rinsed with cold buffer containing 20 mM Tris (*p*H 8.0), 137 mM NaCl, 1 mM CaCl$_2$, 0.5 mM MgCl$_2$ and 10% glycerol (v/v), and then lysed in the same rinsing buffer containing 1% NP40 (v/v). After incubating the cells at 4 °C for 20 min, nuclei were removed by centrifugation at 2,000 r.p.m. for 10 min in an IEC centrifuge. To the supernatant from an equivalent of 2×10^7 cells was added 10 µl of 14b antiserum or nonimmune serum and 40 µl of protein A–Sepharose CL-4B (Pharmacia) which had been equilibrated in rinsing buffer containing NP40 and glycerol. After 2 h in the cold with constant mixing, the Sepharose beads with bound antibody were pelleted by low speed centrifugation (400 r.p.m. for 30 s in an IEC centrifuge), and then washed 3 times with 100 mM Tris (*p*H 8.0) containing 200 mM LiCl and 0.1% 2-mercaptoethanol (v/v), and once with 1 mM Tris (*p*H 7.0) containing 150 mM NaCl. The final pellet was resuspended in 50 µl of 40 mM sodium acetate (*p*H 6.0) containing 10 mM MgCl$_2$ and 7.5 µM [γ-^{32}P] ATP (specific activity 250 Ci mmol^{-1}; NEN), and the mixture was incubated for 10 min at 37 °C. The reaction was terminated by the addition of 100 µl of standard double-strength SDS sample buffer[12] and the immune complexes were eluted from the Sepharose beads by boiling for 2–3 min. The beads were removed by low-speed centrifugation and equal volumes (25 µl) of supernatant were then analysed by SDS-PAGE using a 16% polyacrylamide slab gel. The gel was stained with Coomassie brilliant blue, dried, and an autoradiograph was prepared using Kodak RP X-Omat film. Mock-infected cells treated with 14b (*a*) or nonimmune (*b*) serum. Wild-type Ad 5-infected cells treated with 14b (*c*) or nonimmune (*d*) serum. Cells infected with Ad 5 group II host range mutants hr 6 (*e*) and hr 50 (*f*) treated with 14b antiserum. Cells infected with Ad 5 group I host range mutants hr 1 (*g*) and hr 3 (*h*) treated with 14b antiserum. Coomassie blue-stained gel of wild-type-infected cells treated with 14b antiserum (*i*). ^{35}S-methionine-labelled Ad 5 virion proteins are included as molecular weight markers.

However, studies carried out by Collett and Erikson with the RNA tumour virus Rous sarcoma virus (RSV) suggested that the RSV transforming protein, src, either possesses or is associated with protein kinase activity[1]. Using antiserum directed against virus-coded proteins, it was found that phosphorylation of the heavy chain of the antibody occurred when immunoprecipitates containing src protein were incubated with [γ-^{32}P]ATP. No such protein kinase activity was observed in immunoprecipitates using uninfected cell extracts, extracts from cells infected with transformation-defective virus, or control nonimmune serum. These data suggest that alterations in protein phosphorylation may regulate RSV transformation. This concept is quite appealing, as protein phosphorylation is already known to have an important regulatory role in several cellular processes[2–4]. We show here that antiserum directed against tumour antigens of a DNA virus, human adenovirus type 5 (Ad 5), will also immunoprecipitate protein kinase activity. Further, immunoprecipitates from cells infected with transformation-defective host range mutants of Ad 5 contain reduced levels of protein kinase activity.

We have used an approach similar to that of Collett and Erikson to determine if the transforming gene products of Ad 5 possess protein kinase activity. Antiserum against viral transformation proteins was obtained from hamsters bearing tumours induced by Ad 5-transformed hamster cells. The transformed cells, designated 14b, contain 40% of the left end of the Ad 5 DNA molecule[5] and transcribe 7% of the genome near the left end[6]. This region of the viral DNA molecule is necessary and sufficient for oncogenic transformation[7,8]. Thus, antiserum prepared against 14b cells should contain antibodies directed against only the transforming proteins of Ad 5.

Cytoplasmic extracts were prepared from Ad 5-infected and mock-infected human KB cells and these were immunoprecipitated with immune or control nonimmune serum, using essentially the procedure of Schaffhausen *et al.*[9]. Washed immune complexes were then incubated in the presence of [γ-^{32}P]ATP and the samples were analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Studies were also carried out using extracts from KB cells infected with host range mutants of Ad 5. These mutants have been shown to fall into two complementation groups, I and II (ref. 10), both of which are transformation defective[11].

Figure 1 shows an autoradiograph of immunoprecipitates incubated with [γ-^{32}P]ATP. With wild-type Ad 5-infected cell extracts and immune serum (Fig. 1*c*), a major ^{32}P-labelled species was observed which co-migrated with the heavy chain of IgG (see Fig. 1*i*). Several minor labelled bands of higher molecular weight were also observed, and additional labelled species appeared with prolonged exposure of the gel. Incorporation into the light chain of the antibody was not detected. Little incorporation was observed with extracts from wild-type-infected cells precipitated by nonimmune serum (Fig. 1*d*), or with extracts from mock-infected cells (Fig. 1*a*, *b*). Counts on bands excised from the gel indicated that the amount of ^{32}P incorporated into the heavy chain of IgG was 14 times greater with infected cell extracts and immune serum than with controls. Similar experiments with the host range mutants hr 6 and hr 50 of group II (Fig. 1*e*, *f*, respectively), and hr 1 and hr 3 of group I (Fig. 1*g*, *h*, respectively) gave levels of incorporation into the heavy chain of IgG which were reduced compared to wild-type Ad 5 (30–60% of wild type).

With immunoprecipitates from wild-type-infected cell extracts, the reaction was very rapid, maximum incorporation into IgG occurring within 1 min at 37 °C. No incorporation of ^{32}P into IgG was observed when the immune complex was heated at 100 °C for 2 min before incubation with [γ-^{32}P]ATP.

As high levels of endogenous protein kinase activity exist in all mammalian cells, the activity found in immunoprecipitates from Ad 5-infected cells could be due to nonspecific entrapment during formation of the antigen–antibody complex. To exclude this possibility, an unrelated antigen–antibody complex (human IgG and sheep anti-human IgG) was formed in an extract from Ad 5-infected cells. The complex was immunoprecipitated and incubated with [γ-^{32}P]ATP. No incorporation of ^{32}P into IgG was observed in these conditions (data not shown). Thus, it is unlikely that the Ad 5-associated protein kinase activity resulted from entrapment of cellular enzymes, as the amount of antigen–antibody complex in this control study was far in excess of that obtained using infected cell extracts and immune serum. Results from another experiment suggest that the protein kinase activity found in immunoprecipitates from infected as opposed to control cell extracts was not due to the presence of vastly different levels of total enzyme activity in the two preparations: cytoplasmic extracts (containing equal amounts of protein) were incubated with [γ-^{32}P]ATP in a reaction mixture similar to that described in the legend to Fig. 1 and the amount of protein kinase activity was measured using endogenous substrates (as described in ref. 12). Ad 5-infected cells contained only 1.4 times more total activity than uninfected cells.

Our results are similar to those obtained with RSV[1]. The data suggest that an Ad 5 transformation-specific protein either is a protein kinase or is associated with one. Whether this activity is due to a virus-coded or virus-induced cellular enzyme is at present not clear. However, because the antiserum was raised in hamsters using transformed hamster cells and the immunoprecipitation experiments were carried out with infected human cell extracts, it seems more likely that it is a virus-coded function. The immune antiserum binds specifically to at least two Ad 5-induced polypeptides, of molecular weights of 58,000 and 10,500 (refs 13, 14), as well as other minor species (N.J.L., unpublished). Localisation of protein kinase activity to a specific polypeptide will require further studies. With transformation-defective host range mutants, enzyme activity was reduced, suggesting that these mutants are at least partly deficient in this function. Furthermore, some of the activity obtained with the mutants may be due to leakiness caused by the high multiplicity of infection.

It is now apparent that protein kinase activity is associated with the transforming proteins of both an RNA and a DNA tumour virus and thus alterations in protein phosphorylation may be a general mechanism by which all tumour viruses induce cellular transformation.

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Kinetics of *in vitro* reconstitution of oligomeric enzymes by cross-linking

THE reconstitution of oligomeric enzymes from their denatured state is a consecutive process consisting of folding and association steps. In the case of multimeric enzymes the pathway of reassociation and its correlation with the occurrence of enzymatic activity is an open question. The combined use of cross-linking and SDS-polyacrylamide gel electrophoresis allows the assembly of multimeric enzymes to be followed quantitatively.

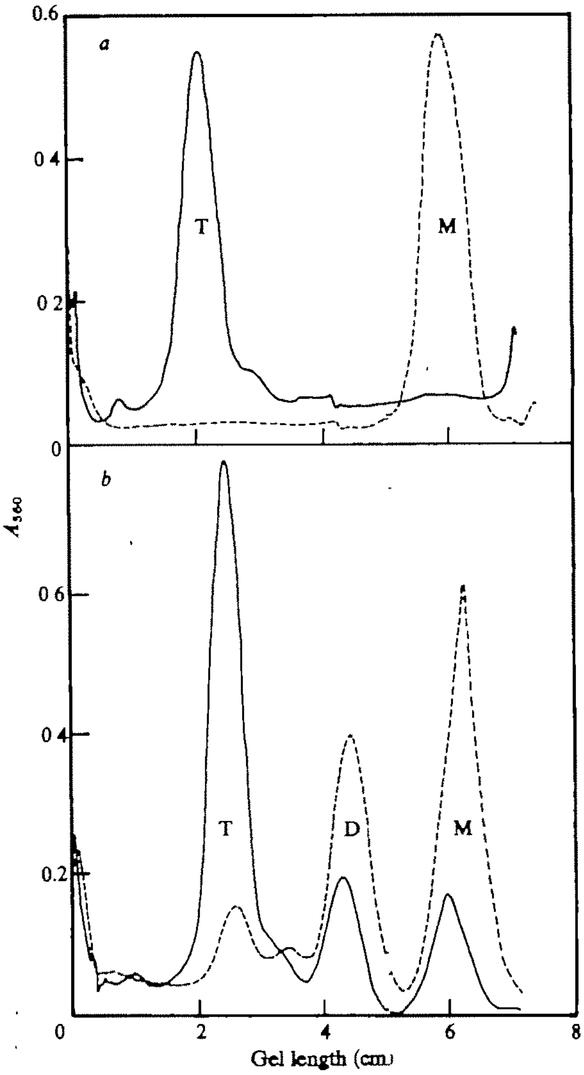

Fig. 1 SDS-polyacrylamide gel electrophoresis of cross-linked LDH-M_4. Cross-linking by 2 min incubation in 0.3–0.4 mM glutaraldehyde, 20 °C, was stopped by addition of 15–25 μM SDS; unreacted bifunctional reagent was inactivated by 3–4 mM hydrazine The solutions were heated for 10 min (100 °C) and subsequently stored for 2 h at 20 °C. For concentrating, the protein solutions were dialysed for 24 h against 20% polyethyleneglycol before electrophoresis (5% polyacrylamide gel containing 0.15% SDS). The gels were stained for 2 h with 0.1% Coomassie blue R 250 in 50% trichloroacetic acid, and destained in 7% acetic acid plus 5% methanol. 0.2 M phosphate buffer, *p*H 7.6, plus 5 mM EDTA was used throughout. The gels were scanned at 560 nm. Correlation of the bands to the tetrameric (T), dimeric (D) and monomeric (M) fractions of LDH was achieved by calibration with proteins of defined molecular weight (dimeric and monomeric human serum albumin, monomeric LDH and lysozyme). *a*, Cross-linking of native tetramers (solid line) and SDS-denatured monomers (dashed line); monomer concentration, 81 nM. *b*, Cross-linking of reactivating LDH after acid dissociation at *p*H 2.3. Reactivation at 20 °C by dilution at *p*H 7.6, final monomer concentration, 343 nM. Cross-linking after 210 s (dashed line) and 1 h (solid line) of reactivation and reassociation.

We report here that by applying this technique to a study of lactic dehydrogenase from pig muscle, we have shown that tetramer formation parallels reactivation of the enzyme.

Nascent polypeptide chains fold spontaneously to defined three-dimensional structures, without additional information beyond the specific amino acid sequence of the given protein and its aqueous environment. In addition, protein molecules above a critical chain length tend to associate to form stoichiometrically and geometrically well defined quaternary structures. In this

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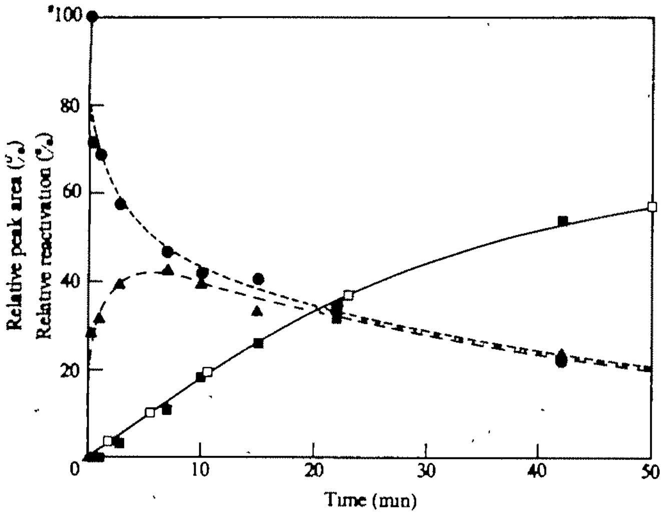

Fig. 2 Determination of the kinetics of reassociation of LDH-M_4 by cross-linking. Experimental details as described in Fig. 1. Reassociation (20 °C) at 170 nM monomer concentration analysed by drawing aliquots at defined times and fixation of the association products by cross-linking. Percentage of monomers (●), dimers (▲) and tetramers (■) defined by the respective peak areas relative to their total amount. Reactivation (□), as determined by testing aliquots at defined times in standard test conditions, was calculated relative to the final extent of reactivation (75% after 6 d).

connection, folding and association can be considered as the final stages of protein biosynthesis. The timing of the two processes must be coordinated because the correct assembly of subunits requires proper folding to defined three-dimensional structures. In this sense the primary function of 'folded monomers' is self-assembly[1].

For single-chain proteins a variety of experimental approaches has allowed the identification of intermediates along the folding pathway[2]. These *in vitro* studies have mostly been aimed at the prediction of three-dimensional structures from the amino acid sequences and the elucidation of the mechanisms dominating the folding process *in vivo*[2–4].

In multi-chain proteins, folding is inherently correlated with the association of subunits. Besides the folding of the isolated polypeptide chains, additional transconformation reactions occur after the association of the native quaternary structure has taken place. Recent analyses of the reactivation kinetics of several oligomeric enzymes have demonstrated that the reassociation process is a prerequisite for conformational transitions leading to the complete restoration of the native enzymatic properties[5–8]. The corresponding reactivation relaxations obey a simple kinetic mechanism consisting of two consecutive first order and second order reactions[6,8,9]. The assignment of the respective rate-limiting steps to certain elementary processes in the overall transconformation–association reaction has not been feasible so far. It would require the quantitative determination of the kinetics of association, for example, by light scattering measurements, which is not practicable because of the unfavourable signal-to-noise ratio at low enzyme concentrations and the difficulties connected with a quantitative population analysis.

Therefore, we developed a method of analysing the association kinetics of oligomeric enzymes by cross-linking the refolding subunits with a bifunctional reagent (glutaraldehyde), and subsequent separation of the various association products by SDS-gel electrophoresis. The method allows the detection and quantitative evaluation of intermediates of association even at very low enzyme concentrations. The kinetics of formation and disappearance of these intermediates can be analysed by taking aliquots of the enzyme solution at defined times during the process of reconstitution. Parallel measurements of the regained enzymatic activity in identical conditions were carried out to correlate the kinetics of reassociation and reactivation. The determination of the kinetic constants of the latter reaction may reveal whether or not subunits as intermediates of reconstitution represent catalytically active entities.

We used lactic dehydrogenase (LDH-M_4) from pig skeletal muscle (Boehringer). The different isoenzymes of mammalian LDH are composed of four subunits, the structural and catalytic properties of which have been investigated in great detail[10]. The hydrodynamic and spectral properties of the dissociated, inactive and partially unfolded monomers, present at *p*H 2.3, have been described previously[11,12]. After renaturation, the reactivated tetramers were found to be indistinguishable from the native isoenzymes by testing for a variety of physicochemical and enzymatic parameters[5,11,12].

The reactivation of both isoenzymes (LDH-M_4 and H_4 from pig) was found to be dominated by second order reactions; slightly sigmoidal relaxations (especially for LDH-H_4) suggest, however, that first order reactions are involved to a certain extent[8,11–13]. The overall process can be quantitatively described as a consecutive uni-bimolecular reaction sequence. For both isoenzymes, the respective rates of reactivation are not influenced by their coenzyme, NADH or NAD^+ (refs 5,14).

In the present experiments, LDH-M_4, after denaturation at *p*H 2.3, was renatured at *p*H 7.6 in the absence of cofactors or substrates. At specified times after dilution, aliquots were analysed with respect to their particle distribution and the regeneration of enzymatic activity. To ensure fast and quantitative intramolecular cross-linking of associated particles, a high molar excess (10^5–10^7) of glutaraldehyde was used. To prevent intermolecular cross-linking, the reaction was stopped after a constant reaction time of 2 min by addition of 5% SDS and inactivation of excess glutaraldehyde by the addition of hydrazine[15].

As Fig. 1*a* shows, optimum conditions gave 100% cross-linking and fixation of native enzyme in its tetrameric state, whereas cross-linking of the SDS-dissociated enzyme yielded only monomers. No high molecular weight products were formed by inter-molecular cross bridges in either of the blank experiments. Therefore, cross-linking and subsequent SDS electrophoresis can be used to determine relative amounts of the different states of association present at various times during reassociation (Fig. 1*b*). The kinetic traces for the decrease of the concentration of monomers and the formation of dimers and tetramers show that within the range of experimental error, tetramer formation clearly parallels the time course of reactivation (Fig. 2).

The identification of monomers, dimers and tetramers during reassociation (Figs 1*b*, 2) shows that at least two reactions are rate limiting in the overall process of reconstitution. Considering the fact that the reactivation kinetics for LDH-M_4 (apart from an insignificant contribution of rapid first order transconformation reactions) can be described by a single second order rate constant[8,11], one may exclude the possibility that two consecutive bimolecular reactions are rate determining for reactivation and tetramer formation. This means that only one of the reactions which is rate limiting for the formation of dimers from monomers, or of tetramers from dimers, is determined by an association process; the other must be of first order[16].

From our data it is not possible to show if the formation of dimers or tetramers is governed by the association reaction. This can only be clarified by analysing the formation of dimers and tetramers at various initial concentrations of monomers. That reactivation and tetramer formation take place together clearly shows that those dimers which accumulate during reassociation do not possess appreciable catalytic activity. This does not rule out the possibility that active dimers occur as intermediates of reactivation. Whether a kinetic model consisting of two consecutive unimolecular and bimolecular reactions is sufficient to describe the observed patterns of dimer and tetramer formation, or whether more sophisticated models, including rapid equilibria, are necessary, cannot yet be decided.

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Formation of a new fluorophore on irradiation of carboxymethylated D-glyceraldehyde-3-phosphate dehydrogenase

BECAUSE of its physiological importance, allosteric properties and ready availability, D-glyceraldehyde-3-phosphate dehydrogenase (G3PDH) is one of the most thoroughly studied of the dehydrogenases. We report here a study of the ligand binding properties of G3PDH. We observed that when active site carboxymethylated G3PDH was irradiated with near ultraviolet light in the presence of NAD^+, a new fluorophore was formed.

A six-times recrystallised G3PDH from rabbit muscle was modified with iodoacetate until less than 5% of the original activity remained. The reaction mixture was then passed through a Sephadex G-50 column to remove excess iodoacetate. Determination of the sulphhydryl groups present before and after iodoacetate treatment with 5,5′-dithio-bis(2-nitrobenzoic acid) showed that only one sulphhydryl group per subunit was modified—this is presumably Cys-149 at the active site[1]. When the active site-carboxymethylated enzyme was irradiated in the presence of excess NAD^+ with a 150-W xenon lamp for 6 min, a new fluorophore was formed. The excitation spectrum of the new fluorophore shows peaks at 290 and 325 nm whereas the emission maximum is at 410 nm (Fig. 1). The photochemical reaction leading to the formation of the new fluorophore appears to be quite specific. Neither the holoenzyme nor the iodoacetamide- or sodium tetrathionate-modified enzyme can form the new fluorophore. At much higher concentrations, $NADP^+$ and NMN^+ can replace NAD^+ to form new fluorophores with emission maxima at 410 and 385 nm respectively, whereas AMP, ATP or ADPR are completely ineffective. These results suggest that the nicotinamide ring and the carboxyl group introduced at the active site Cys-149 are involved in the formation of the new fluorophore.

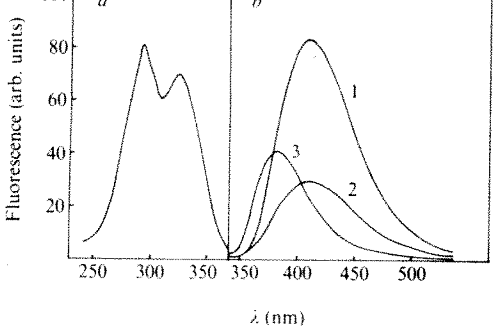

Fig. 1 Excitation and emission spectra of the fluorophores formed on irradiation of carboxymethylated G3PDH with nicotinamide nucleotides. *a*, Excitation spectrum of the fluorophore formed with NAD^+. *b*, Emission spectra of the fluorophores formed with NAD^+(1), $NADP^+$(2) and NMN^+(3). Enzyme concentration in all cases, 3 μM.

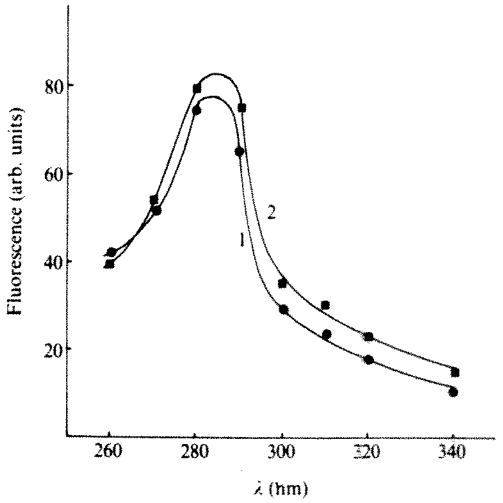

Fig. 2 Photochemical action spectrum for the formation of the NAD^+ fluorophore. Portions of carboxymethylated G3PDH, 3 μM and NAD^+, 33 μM in 0.1 M phosphate buffer, *p*H 6.7 were directly irradiated with lights of different wavelengths through the excitation monochromator of a MPF-4 spectrofluorometer at a slit width of 10 nm for 20 min each to give curve 1. The excitation spectrum of a concentrated rhodamine B solution was then scanned. The fluorescence intensity of this solution at each wavelength of excitation is directly proportional to the quantum input at this wavelength and can be used for the correction of curve 1 to give the true photochemical action spectrum for the formation of the new fluorophore (curve 2).

The new fluorophore is tightly bound to the enzyme protein as shown by the following experiments. Treatment with excess activated charcoal removed all the NAD^+ added in excess and raised the ratio of A_{280} to A_{260} to 1.4–1.5, but failed to remove the fluorophore from the enzyme protein. After passage through a Sephadex G-50 column, only the protein fraction was fluorescent, the NAD^+ fraction showed no significant fluorescence. Furthermore, ethanol at concentrations greater than 60% also precipitated the enzyme protein together with the fluorophore.

When carboxymethylated G3PDH was irradiated with monochromatic light in the presence of NAD^+, the photochemical action spectrum obtained (Fig. 2) showed a broad maximum in the 280–290 nm region. Absorption by tyrosine residues decreases sharply at wavelengths longer than 280 nm, so our findings implicate strongly the excitation of tryptophan residues in the formation of the new fluorophore.

With excess NAD^+ removed by charcoal adsorption, the irradiated enzyme shows a broad absorption band at 325 nm due to the new fluorophore. This overlaps with the 295 nm excited protein fluorescence at 335 nm due to tryptophan residues. During the course of irradiation, with the gradual appearance of the new fluorescence band at 410 nm, the tryptophan fluorescence was quenched as shown in Fig. 3. Determinations with *p*-*N*-dimethylaminobenzaldehyde showed that no destruction of tryptophan residues had occurred. The quenching of tryptophan fluorescence is, therefore, due to a radiationless energy transfer from tryptophan to the new fluorophore. As both the A_{280}/A_{260} ratio and the amino acid composition of the irradiated enzyme seem to suggest that the photochemical formation of the new fluorophore may also be a 'half-of-the-sites' reaction,

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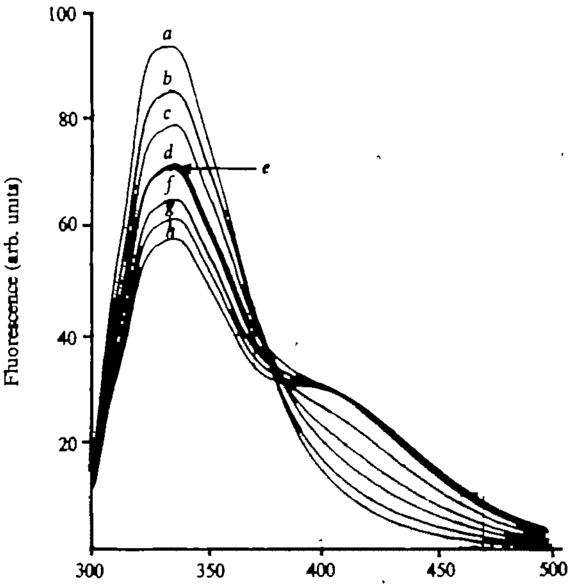

Fig. 3 Quenching of tryptophan fluorescence during the formation of the new fluorophore. Carboxymethylated G3PDH, 3 μM and NAD$^+$ 33 μM in 0.1 M phosphate buffer, *p*H 6.7 was irradiated in the MPF-4 spectrofluorometer with an exciting light of 290 nm and a slit width of 20 nm. The emission spectra with the exciting wavelength also at 290 nm were scanned at *a*, 0, *b*, 1.5, *c*, 3; *d*, 6; *e*, 9; *f*, 12; *g*, 15 and *h*, 20 min.

this energy transfer involves probably only two of the four subunits. Calculations based on the Förster equation with the orientation factor K^2 taken as 2/3 give a distance between tryptophan and the new fluorophore of 15.79 Å. As each subunit of the rabbit muscle enzyme has three tryptophan residues, this probably represents a weighted average value of the distances between the three tryptophan residues to the new fluorophore. This value can be compared with the results of X-ray crystallographic analysis of the lobster[3] and bacterial[4] enzymes.

The formation and the fluorescence of the new fluorophore were found to be sensitive to conformational changes of the enzyme protein. Sodium dodecylsulphate (SDS) at a weight ratio of 1:1 with the enzyme led to a 50% decrease in the fluorescence intensity at 410 nm. However, even more marked was the effect of SDS on the formation of the new fluorophore. As shown in Fig. 4, the presence of SDS at a weight ratio of 0.5 during irradiation was enough to decrease the 410 nm fluorescence formed by 75%. It has been reported[5] that SDS at a weight ratio to G3PDH of 0.5 loosened its tertiary structure and at a ratio of 1 dissociated this enzyme. Our own studies by difference spectroscopy and protein fluorescence also showed that with increasing SDS to enzyme ratios, the internal tryptophan and tyrosine residues of G3PDH were gradually exposed. The great sensitivity of the formation of the new fluorophore seems to suggest that the photochemical reaction leading to its formation has strict requirements on the spatial relationship of excited tryptophan residue(s), the carboxymethyl group introduced at Cys-149 and the nicotinamide ring.

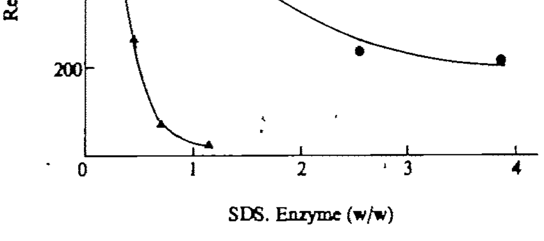

Fig. 4 Effect of sodium dodecylsulphate on the formation and intensity of the new fluorophore. Curve 1 shows the effect of SDS present during irradiation on the formation of the 410 nm fluorophore. Curve 2 shows the effect of SDS added after the formation of the new fluorophore on its fluorescence intensity. Relative fluorescence intensity plotted against the weight ratio of SDS to the enzyme. Final concentration of the enzyme was 2 μM with 22 μM NAD$^+$ in 0.1 M phosphate buffer, *p*H 6.7.

Further studies are needed to establish the chemical nature of the new fluorophore. At present, we can say that it is tightly, probably covalently, bound to the enzyme protein and its spectral and fluorescence properties closely resemble a series of addition compounds[6] of NAD$^+$ including NADH.

In spite of some earlier suggestions[7,8], very little is known about the role of tryptophan residues in G3PDH. Our results demonstrate the close relationship between tryptophan, Cys-149 and the nicotinamide ring and suggests the need for close examination of this problem.

Further details of this work will be published elsewhere[9].

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Errata

In the letter 'Photographic amplification of faint astronomical images' by D. F. Malin, *Nature* **276**, 591–593, the figures have been transposed. Figure 1 is shown above Fig. 2 legend and Fig. 2 above Fig. 1 legend.

In the article 'Olivine fractionation equations for basaltic and ultrabasic liquids' by T. H. Pearce, *Nature* **276**, 771–774, equation (12) should read:

$$\log\left(\frac{y}{z}\right)=K\log\left(\frac{x}{z}\right)+\log\left(\frac{y_0}{x_0}\times\frac{z_0^{k-1}}{x_0^{k-1}}\right)$$

equation (14) should read:

$$\log\left(\frac{\mathrm{FeO}}{\mathrm{Al_2O_3}}\right)_{\mathrm{l}}=K\log\left(\frac{\mathrm{MgO}}{\mathrm{Al_2O_3}}\right)_{\mathrm{l}}+\log\left(\frac{\mathrm{FeO}}{\mathrm{MgO}}\right)_{\mathrm{P}}\times\left(\frac{\mathrm{Al_2O_3}}{\mathrm{MgO}}\right)_{\mathrm{P}}^{k-1}$$

In the article 'Analysis of ^{36}Cl in environmental water samples using an electrostatic accelerator' by D. Elmore *et al.*, *Nature* **277**, 22–25, the last sentence in the summary should read: 'For each sample less than 70 mg of AgCl were used, requiring 1–5 litres of water.' Line 19 on page 23 should read 'with mass spectrometers lies in the difficulty of separating...' In line 41 for 'from' read 'with'. In line 47 for ref. 18 read ref. 17. In Table 1 the units in the last three columns should read: (d.p.m. per kg Cl); (mg l^{-1}); (×10^{-6}, atoms l^{-1}). An extra line should be added to the table:

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In line 31 in the right column for 'neutral' read 'natural' and in line 54, for 100-mg read 20-mg.

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reviews

Making sense of man's universe

Garrett Hardin

The Little Universe of Man. By C. D. Darlington. Pp. 307. (George Allen and Unwin: London, 1978.) £6.95.

THIS is a biologist's attempt to make sense of the world's past, to foresee its probable future and, by putting foresight into words, possibly to influence what comes.

On what basis shall we appeal to people to alter their actions? "As the teachings of Adam Smith and Robert Malthus, Karl Marx and Charles Darwin agree, the animal principle of the self-interest of individuals, often, as we know, enlightened and extended, is the only motive to be infallibly relied on in the conduct of human affairs". It is psychologically necessary to believe in the rightness of one's action if that action is to be pursued with the fervour required to melt away opposition; out of this necessity springs the paradox of self-interest creating self-deception.

Does self-deception have survival value? It depends on the stage of ecological maturity of society. "'As the resources of the earth become more depleted and populations both more crowded and more deprived, the greater will be their effort to pass the problem on to the next generation. Self-deception will become more persuasive. Optimism will take hold. Those who remember 1914 and 1939 will also remember that these smaller calamities were produced by optimists but foreseen by pessimists. Indeed before these events an optimistic temperament had seemed to confer a permanent selective advantage in human evolution. It is not so now".

For the extreme example of an approach that will not solve the world's problems Darlington cites witchcraft: "The profession of witch-doctor dominates Negro life in Africa, creating a religion whose redeeming features we seek in vain". Despite justifiable criticisms that can be levelled against it the European approach to reality is the world's last and best hope: "The world is indepted to Europe for two great achievements which have transformed human life in the last 500 years. One is the control of nature which has led to our present crisis. The other is the understanding of nature and of man which is our means of escape from this crisis".

At every stage in our progress in understanding ourselves our will to self-deception erects taboos capable of delaying understanding for generations. In the English-speaking world the idea of man as an animal was under a taboo from about 1819 to 1859; human sexuality continued under taboo until about 1930; and the dispassionate discussion of the existence and consequences of innate human differences is still seldom possible. To a geneticist, of course, it is inconceivable that innate, heritable differences could not exist, and it is hard to believe that none of these is humanly important. A Darwinian biologist (and what other can there be?) looks for selection everywhere. Darlington's book is permeated with the Darwinian outlook. It takes only a moment for a perceptive biologist to postulate selective adaptations that may require years to validate. Darlington postulates many, so his book should stimulate many controversies.

The author is not unsympathetic to the currently reigning taboo: "Innate hereditary or genetic differences must not be admitted between individuals or groups, between classes or races, or even between the sexes. But above all what must not be discussed, what must be rejected, are differences in the foundations of human behaviour, the study of brains, of instincts, and of intelligence. These foundations are complex and happily concealed from the public view. They must remain concealed. In a world already overcrowded and over-troubled they might cause more trouble".

A great source of the ever-verdant controversy of nature versus nurture is the awkward fact of causal circularity, sometimes called the "Baldwin effect." First we select our inventions and then our inventions select us. Equestrian cultures undoubtedly selected for the ability to manage horses, which not all men have. Agriculture and the capital accumulation that it made possible selected for the willingness to engage in sustained, hard work and for a new form of competitiveness between tribes. The increase in the supply of food brought about by agriculture did not set supply and demand permanently in balance: it merely moved the instability to a higher population level and rewarded those who could successfully migrate into new and relatively unexploited environments. Language selected for those who could manipulate language—and their fellow-men—most competently. This fact justifies the overwhelming emphasis on language in intelligence tests; it also explains the passion invested in a particular language. "People have treated the survival of their language in speech and writing as a matter of life and death. And they have been right in doing so since language guarantees the survival of the breeding community".

Trying to make sense of the whole universe of man, as Darlington does, is an ambitious undertaking, scarcely mitigated by calling it a *Little Universe*. Every thoughtful person will find many points of disagreement with the author. The beauty of it is that the points of disagreement are easy to find, for Professor Darlington's exposition is crystal clear. The result is a splendid book for stimulating important discussions. ☐

Garrett Hardin is Professor Emeritus in the Department of Biological Sciences at the University of California at Santa Barbara.

Coral reef research

Coral Reefs: Research Methods. Edited by D. R. Stoddart and R. E. Johannes. Pp. 581. (UNESCO: Paris, 1978.)

THE study of coral reefs, earlier largely sustained by occasional expeditions, sprang suddenly into the fullest vigour in the Marshall Islands in 1945. This was in preparation for the atom bomb tests that began in the following year in Bikini and then Eniwetok atolls. Preliminary studies were exhaustive in their oceanographical and descriptive ecological content, and as a result of unprecedently deep boring, the validity of Darwin's postulates (if not their universal application) was established.

This impetus was sustained by the Coral Atoll Program of the Pacific

Science Board of the National Academy of Sciences of the USA with many of the results appearing in the *Atoll Research Bulletin* Important guidance was provided in the *Handbook of Atoll Research* published in 1953 by F. R. Fosberg and M.-H Sachet of the Smithsonian Institution who also edited the *Bulletin*.

Today the subject matter has proliferated widely. The mapping of reefs presents unique problems owing to geographical or seasonal inaccessibility and constantly changing pattern. New methods have developed for drilling, for determination of the sediments into which the reef mass disintegrates and for the local determination of sea level. Evaluation of a fauna and flora living on largely three-dimensional reefs with a territorial fish population presents problems not encountered in temperate seas.

In striking contrast to the surrounding oceanic deserts, atolls are oases of high productivity. But they are fragile systems without any sustaining reservoir of nutrients and phytoplankton, dependent on continuous re-cycling Everything removed, notably fish, affects total production. The influence of the algal zooxanthellae present in all hermatypic corals on both calcification and nutrition awaits final evaluation

Many working on these problems have devised new methods of attack, and new techniques such as aerial photography have appeared The whole subject has been critically reviewed by Working Group 35 of the Scientific Committee on Oceanic Research of UNESCO which is responsible for this volume with 43 contributions. These are grouped under Morphology and Structure, Biotic Distributions, and Energy and Nutrient Flux, their value the greater because the two first are edited by D R. Stoddart and the third by R E. Johannes, respectively the leading workers on the geomorphology and on the productivity of reefs

Both field workers and those more theoretically interested in the problems of coral reefs will find this book of the greatest help; it will also have permanent value as a record of the current state of enquiry.

C. M. Yonge

Sir Maurice Yonge is Honorary Fellow in the Department of Zoology, University of Edinburgh, UK.

Marine micropalaeontology

Introduction to Marine Micropaleontology. Edited by B. U. Haq and A. Boersma. Pp.376. (Elsevier: New York and Oxford, 1978.) $24.

It is fifteen years since the English translation of Pokorny's *Principles of Zoological Micropalaeontology* was published. Since that time there has been no new general text in micropalaeontology, despite the major contribution that the study of microfossils has continued to make in world exploration for oil and gas, and the successive achievements of the Deep Sea Drilling Project. It is perhaps not as well recognised as it ought to be that confirmation of the hypothesis of oceanfloor spreading and plate tectonics in the ocean basins is almost totally dependent on micropalaeontological evidence. Also the general application of 'absolute' ages to marine geological events is heavily dependent on the precision with which micropalaeontological biozones have been related to otherwise relatively isolated radiometric and palaeomagnetic determinations. Latterly too, in association with isotopic analysis, micropalaeontology has begun to yield significant new insights into the periodicity and chemistry of major oceanic changes that are themselves linked to worldwide variations in the planetary environment.

During this period of development knowledge of the various plant and animal microfossil groups has been vastly extended, new numerical methods introduced, and new technologies, such as scanning electron microscopy, adopted. Perhaps it is because of all this endeavour that no micropalaeontologists have had time to compile a review of the current state of their science. Here at last, however, Bilal Haq and Anne Boersma have persuaded a distinguished group of specialists to contribute to a pre-arranged pattern, a series of up-to-date accounts of the groups which they study. Thus, we have separate chapters on foraminifera, calcareous nannoplankton, ostracodes (by Pokorny himself), pteropods, calpionellids, calcareous algae and bryozoa—all mainly of calcareous composition; radiolaria, diatoms, silicoflagellates—all siliceous; and conodonts, dinoflagellates, spores and pollen and chitinozoa—of phosphatic and organic composition.

Each account gives an outline of the biology, taxonomy and stratigraphical distribution of the respective groups, but more interestingly usually goes on to consider their palaeoecology and contribution to palaeo-oceanic problem-solving They therefore serve at the same time the prosaic purpose of imparting technical information to the would-be specialist, and also stimulate an interest in problems of more general concern that can be approached by way of micropalaeontology. Naturally with so many different authors contributing the quality is sometimes variable, and, in spite of the extensive review system adopted, the text (be warned) does contain some unexpected errors Nevertheless the editors are generally to be commended on what they have achieved in terms of uniformity, quality and accuracy. The whole impact of the book is greatly helped by the generous use by all authors of a variety of types of line and half-tone diagrams and illustrations Here is a text that fully realises that endless prose is not always the best way of imparting information and understanding.

B. M. Funnell

B M Funnell is Professor of Environmental Sciences at the University of East Anglia, Norwich, UK

Chemistry and metabolism of vitamin D

Vitamin D Edited by D. E. M Lawson. Pp 433. (Academic: London, New York and San Francisco, 1978.) £20.50; $42 40.

Until the late 1960s vitamin D was generally regarded as being a subject of rather academic interest. It was acknowledged to play a role in intestinal calcium transport; and the main symptom of vitamin D-deficiency, a disorder of bone formation, was attributed not to a direct effect of the vitamin but to the secondary disturbance of calcium metabolism.

The past decade has seen dramatic advances in our understanding of the role of this vitamin. It would now be more appropriate to regard it not as a vitamin but as a metabolite and pro-precursor of a steroid-like hormone. The two hydroxylations, one in the liver and a subsequent one, controlled by parathyroid hormone, in the kidney convert the vitamin to an active form 1,25-dihydroxycholecalciferol. This latter compound acts on intestinal cell nuclei resulting in the induction of synthesis of proteins, including a calcium-binding protein, and leading to an increase in intestinal calcium transport, it is also the most potent stimulator of bone resorption yet discovered The clinical application of this information has been remarkably rapid Less than three years after the discovery of the role of the kidney in the intermediary metabolism of vitamin D an appropriate analogue had been synthesised and applied therapeutically by clinicians involved with the management of patients in chronic renal failure.

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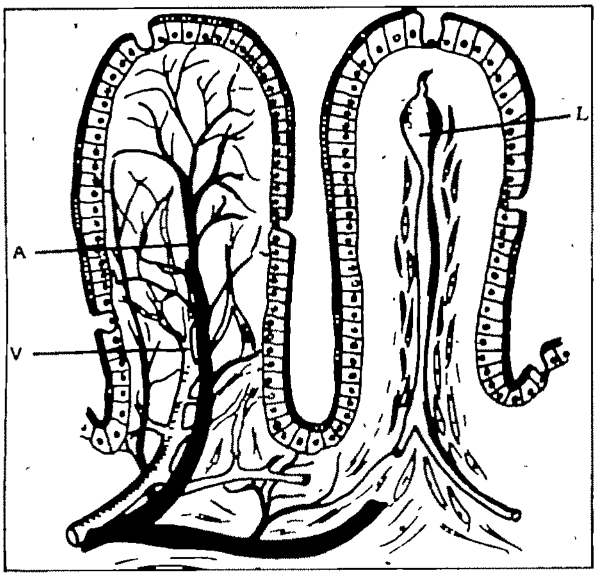

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Proceedings of the 6th L.H. Gray Memorial Conference held at Bedford College, London, in September 1974.
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Reports and discussions of work on the initial shapes of cell survival curves are presented. Major topics included are the biological applications of statistical methods, the construction of mathematical models for cell survival, experimental and theoretical work on oxygen enhancement, fractionation and observations of tumours *in vitro* and *in vivo*.
November 1975 — 412 pages
0471 02513 5 — $35.50/£16.75
Published jointly by John Wiley and Sons Ltd, and the Institute of Physics.

Handbook of Clinical Ultrasound
edited by **M. deVlieger**, *Academic Hospital, Rotterdam.*
The first major work to cover thoroughly the scientific application of ultrasound in all medical specialties. It is composed of ninety-five chapters logically categorized into eight major specialty areas which are individually edited by internationally recognized specialists in their fields. It will provide the ultrasound professional with a sound basis for understanding this increasingly important diagnostic technique. Over 1,000 high-quality illustrations (photographs, scans, ultrasonic images, charts, tables, and diagrams) are included along with an extensive selection of references for further investigation.
December 1978 — 990 pages
0471 02744 8 — $85.00/£42.50

Medical Anthropology
by **G.M. Foster** and **Barbara G. Anderson**,
both of the University of California, Berkeley
A general survey of the field which examines similarities in health-related behaviour in both Western and the developing countries. A cross-cultural perspective emphasizing common elements of medical systems regardless of cultural context is stressed. The book provides comprehensive coverage drawing on pertinent medical sociology as well as anthropology. Also included are sociocultural factors in diet, a comprehensive discussion of medical institutions in the United States and a sophisticated, up-to-date presentation of ethnomedicine.
December 1978 — 368 pages
0471 04342 7 — $18.50/£9.75

Estrogens and Cancer
edited by **S.G. Silverberg** and **F.J. Major**, *both of the University of Colorado School of Medicine, Denver.*
This brings together with epidemiologic, clinical, and pathological data on the multi-faceted relationships between oestrogens and naturally occurring human neoplasms. It features the combined expertise of many eminent contributors, providing a multidisciplinary, one-volume source of current knowledge and future research directions regarding the often-controversial relationship between oestrogens and cancer.
December 1978 — 208 pages
0471 04172 6 — $30.00/£15.85

Non-Invasive Techniques in Cardiology: For the Nurse and Technician
by **A. Benchimol**, *Institute for Cardiovascular Diseases, Phoenix, Arizona*
A concise handbook for all those interested in the technical aspects of non-invasive diagnosis in cardiology. It presents basic guidelines for clinical application of the principles of ultrasound, with in-depth discussion of anatomy and physiology as well as specific disease states. More than 200 illustrations of phonocardiograms, pulse wave recordings, vectorcardiograms, and echocardiograms are included for increased understanding.
December 1978 — 336 pages
0471 04440 7 — $22.40/£11.95

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Despite the frenzy of research activity, particularly over the past five years, we still know remarkably little of the function of the numerous metabolites of vitamin D or their mode of action on target tissues. The complex interrelationship between vitamin D and the other calcium-regulating hormones and the diverse nature of the organs and tissues involved also emphasises the value of a multidisciplinary approach to the research This book is particularly valuable not only because it provides an excellent review of the current state of our knowledge of vitamin D but also because it serves as such a useful reference source of background information for researchers in various areas A number of the contributors to this volume are leaders of the research groups responsible for the major advances in vitamin D research and the various chapters therefore provide an authoritative and overall dogmatically balanced account of the literature and of current views on the many controversial aspects of vitamin D.

The chemistry and metabolism of the calciferols is reviewed in two chapters by Bell and by Holick and DeLuca respectively. Three subsequent chapters by Norman, Wasserman *et al.* and by Lawson consider the intestinal system from different aspects. One quarter of the entire text is devoted to two chapters on bone, by Aaron and by Barnes and Lawson, and a large proportion of these chapters consists of a review of the histology and biochemistry of growth cartilage and bone. Establishing the role of vitamin D in bone formation is clearly one of the main priorities for the next decade and the importance attached to the subject in this book is a reflection of this. The physiology and pathology of vitamin D in man is considered in two more chapters by Stanbury and Mawer, and in the final one Parsons discusses the relationship between it and other calcium-regulating hormones.

Most subjects are covered well but in such a forward-looking book I am surprised that the role of parathyroid and muscle as possible target tissues, and the control of intracellular calcium movement, were not considered in more detail. Overall though this is a valuable contribution to the literature and satisfies a very real need. It will be widely read and appreciated by the large numbers of biochemists, physiologists, nutritionists, clinicians and all others having an interest in calcium and phosphate metabolism

Ian Dickson

Ian Dickson is a member of the Tissue Physiology Group at the Strangeways Research Laboratory, Cambridge, UK.

Centrifugation techniques

Centrifugation: A Practical Approach. Edited by D. Rickwood. Pp. 224. (Information Retrieval: London and Washington, 1978.) Hardback £8; $16; paperback £4; $9.

THE subject matter of this book justifies the publishers' claims that it is an introduction to the technique with an essentially practical basis. It discusses differential pelleting, rate zonal and isopycnic methods and their appropriateness for various purposes with different types of macromolecules, subcellular particles and cells. The operation of all types of rotor, including analytical, is described.

There are numerous suggestions for experiments that contribute to the understanding of principles. The comprehensive details provided embrace even the analytical methods to be used after centrifugation. Some of the warnings included imply that the recommended procedures have been modified in the light of experience. Apart from experiments that involve merely the generation or analysis of gradients, expensive equipment is involved. An unsupervised novice would therefore be well advised not to be too ambitious.

On the whole the authors are successful in choosing a happy medium between the risks of overloading the text with qualifying clauses and of presenting oversimplified statements. At some points the grammatical purist may wince, but there are few slips or printers' errors, although "Pedersen" is consistently spelt "Pederson". There is a useful glossary of terms, but symbols are not invariably standard throughout the book.

With two exceptions the number of references at the end of each chapter has a maximum of ten. This restraint is commendable in a book of this nature, but occasionally results in a misleading impression. For instance, the short section dealing with the hydrostatic pressure of gradients contains only recent references and bears no hint that the existence of pressure gradients in any solution being centrifuged has long been recognised.

As there is a paucity of publications covering the same field at an elementary level, this book should find wide acceptance, especially as the price of the paperback should encourage younger workers to have a personal copy.

P. A. Charlwood

P. A. Charlwood is on the scientific staff of the MRC at the National Institute for Medical Research, London, UK.

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Modification of perceptual systems

Perceptual Modification: Adapting to Altered Sensory Environments. By R. B. Welch. (Academic: New York, San Francisco and London, 1978.) $24.50; £15.90.

THIS excellent book provides the most comprehensive review currently available of research on adaptation of the human senses to rearrangement of the stimulus field. The author has carefully summarised the principal findings of over 500 relevant experiments both published and unpublished, beginning with the late nineteenth century, and including important works completed only during the present year. The research methods used in each experiment are described clearly and succinctly, and are assessed with an objective attitude that is pleasantly refreshing in a field in which evaluative reviews can often be exceedingly biased.

The greater proportion of this book is devoted to examining the diverse types of adaptation to prismatic-induced distortions of the visual field. The various mechanisms that have been proposed to account for the adaptive characteristics of the visual system are presented in detail, and their relative success in explaining the different types of adaptation to visual distortions is reviewed critically. In comparing these mechanisms, the author is careful to make an important distinction between adaptive changes occurring specifically within the visual system and adaptive changes in how vision and movement are coordinated. The failure to make that distinction has often been a source of conflicting results in previous research studies.

The outstanding sections of this book are the reviews on the necessary and sufficient conditions for adaptation to prismatic displacement, adaptation to visual transposition, adaptation to contour distortions, and differences in adaptibility among species and individuals. I was particularly impressed by the logical transition from the review of research on adaptation to inversions of the visual field to the review of research on adaptation to small angular rotations of the visual field. His treatment of these topics was notably insightful and informative; a consequence of an exceptionally clear presentation.

The superior chapter in this book, however, is that on curvature adaptation, in which the author effectively contrasts adaptive changes in visual mechanisms used for processing information about objective shape with those used for processing information about object location. The classical controversy over the role of 'active' as against 'passive' movement in leading to each type of adaptation is also treated most elegantly in that chapter.

If the book has a weakness, it would be the author's commitment to try to make sense out of each and every one of the many diverse findings that he so thoroughly summarises. As he himself points out, there seems to be an almost unlimited number of factors that can contribute to perceptual adaptation. In the final chapter, he proposes a simple theoretical model with the intention being to provide a complete account of perceptual adaptation and thereby resolve numerous controversies in the literature. Although his model does serve well as a descriptive summary of the types of conditions that lead to perceptual adaptation, it really does not provide new insights into what the fundamental underlying processes might be. My suspicion is that there are many such processes, making an adequate model necessarily more complex than the author acknowledges.

This, however, is a minor criticism; in general, the book is an excellent summary of our current state of knowledge about the extent to which perceptual systems can be modified. It is highly recommended not only as a valuable reference for the professional researcher, but also as an informative introduction for the novice. I think the single most impressive feature of the book is the author's skill at organising an overwhelming number of isolated experiments, so as to provide the reader with a coherent account of one of the most confusing areas of visual perception.

Ronald A. Finke

Ronald A. Finke is a Spencer Foundation Predoctoral Fellow in Psychology at the Massachusetts Institute of Technology, Cambridge, Massachusetts.

Catecholamine receptors

Receptor Binding Studies in Adrenergic Pharmacology. By L. T. Williams and R. J. Lefkowitz. Pp. 157. (Raven: New York, $21.45.

THE proposal by Ahlquist thirty years ago that the responses of tissues to catecholamines are mediated by either α- or β-adrenoceptors has formed the basis of the development of a great number of agonist and antagonist drugs for these receptor sites. It has been somewhat surprising therefore, that despite this rich selection of pharmacological probes, reliable techniques to directly identify catecholamine receptors have only been developed over the past three to four years. However, over this latter period a multitude of research papers have been published describing direct labelling techniques, and substantial advances have been made in our understanding of the characteristics and regulation of these important recognition sites.

The authors of this monograph have played major roles in the development of radiolabelled ligand binding techniques for both α- and β-adrenoceptors. It is therefore to be expected that much of this book describes, in considerable depth, work from their own laboratories. The authors have, however, written useful introductory and linking chapters. There are succinct accounts of the pharmacology of adrenergic receptors, theory of ligand–receptor interactions and a historical perspective reviewing some of the frustrations and disappointments that were inherent in the development of these techniques. The chapter on methodological approaches contains a few 'tricks of the trade' that may be useful for those embarking on these techniques. However, it was somewhat disappointing that discussion of atypical binding characteristics such as those associated with negative co-operativity and multiple receptor sites, are dealt with only superficially. Nevertheless, the subsequent chapters, devoted to the pharmacological characteristics of α- and β-adrenoceptor binding sites, are particularly detailed and emphasis is rightly placed on the importance of using radiolabelled agonists as well as antagonists.

The development of adrenergic receptor binding techniques has undoubtedly given us a new insight into the way cells may regulate their responsiveness to catecholamines. A chapter devoted entirely to this topic includes a useful account of the theoretical consequences of 'spare receptors' and the intracellular amplification of extracellular signals. Homologous and heterologous hormonal regulation of adrenergic responsiveness are discussed in some depth, though other aspects such as receptor modulation in developing tissue and genetic influences receive less attention.

Although there is no sign that the initial explosive increase in adrenergic receptor research is saturating, this book nevertheless is a timely review and the lucid style of the authors should ensure that it will become a primary and valuable source of information in adrenergic receptor pharmacology.

S. R. Nahorski

S. R. Nahorski is Lecturer in Pharmacology and Therapeutics in the School of Medicine at the University of Leicester, UK.

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Paul Nettesheim, Chief of Laboratory, Pulmonary Function and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, U.S.A.

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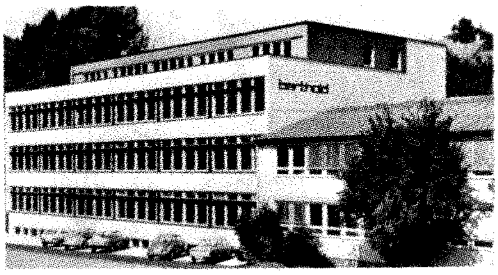
berthold INFORMATION

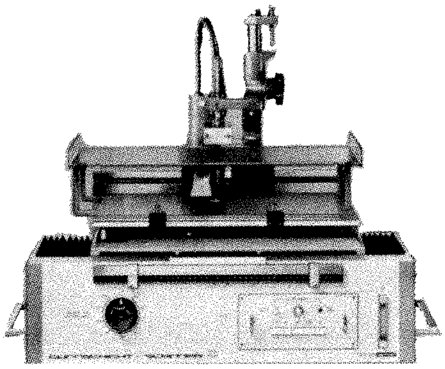
May we introduce ourselves? The name in Berthold



Chances are that you may never have heard of a company with the typically German-sounding name of Laboratorium Prof. Dr. Berthold. The probability, however, is steeply rising that you will encounter one or the other piece of Berthold instruments in any Life Sciences or Medicine Lab handling radioactive isotopes.

Berthold, whose factory is situated at Wildbad (Black Forest) F.R.G., is a fast growing enterprise designing and manufacturing Radiation Measuring Systems for Industry, Science, and Medicine since more than 25 years. Today the company owns branch offices in Belgium, France, Switzerland and Austria and has representatives in nearly30 countries all over the world — the youngest one being Laboratory Impex Ltd. of Twickenham, Great Britain.

It all began with scanners for the thin-layer and paper chromatography. The thin-layer scanner succeeded rapidly in becoming widely known and valued throughout the world. Today there are well over 1000 thin-layer scanners in operation!



Thin Layer Scanner LB 2723

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Other novel and in most cases patented, instrumentation followed: first for radiogas-chromatography, then for column radiochromatography, to be finally followed by the Beta Camera for thin-layer chromatograms.

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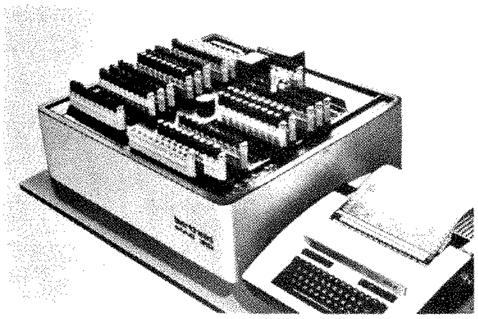

Gamma — Sample Changer LB 310

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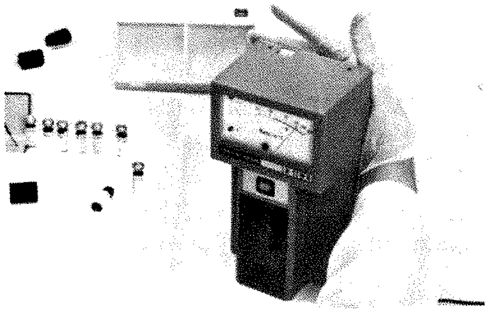
The introduction of the new automatic gamma sample changer was somewhat easier. This was probably a matter of having had the right apparatus at the right time to take advantage of the radioimmuno assay boom. Still, our immediate success came as a surprise — even to us.



Portable Contamination Monitor
LB 1210B

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And there is yet another speciality of Berthold, the radiation protection instruments. A complete line of monitors of most advanced design is offered. As an example only, it may be mentioned that Berthold one year ago introduced the sealed-off, Xenon-filled propertional counter tubes with 100cm^2, 200 cm^2 or 900 cm^2 effective window area which revolutionized completely contamination measuring customs in Nuclear Medicine. If you are interested to get to know further this dynamic company and its products, please contact

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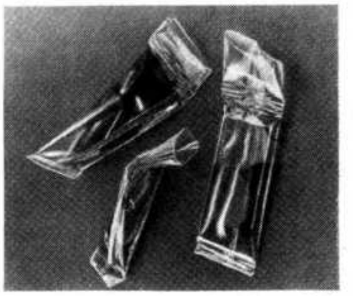
what's new—radioactivity measurement

These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. More detailed information may be obtained by circling the appropriate numbers on the Reader Enquiry Card, bound inside the cover.
A feature on laboratory design and safety will appear in the 15 February issue.

Nalge (Sybron). Nalge offers a new system for scintillation counting based on a new principle: the Filmware tube. Filmware scintillation tubes combine the advantages and eliminate the disadvantages of glass and plastic vials. This system costs $60 to $250 less per 1,000 samples than conventional systems. Filmware tubes are available in two sizes—3 ml and 10 ml. In the Filmware tube, 1 ml of cocktail is distributed vertically for the length of the photomultiplier tube, which therefore gets a good, efficient look at it. In addition, it is possible with the 20 ml vial for the liquid level to go above the height of the photomultiplier tube. The Filmware tube gives optimal exposure to the photomultiplier tube, and often results in greater efficiency than standard vials. Like glass, Filmware tubes allow little if any loss of toluene.


Filmware scintillation tubes from Nalge

A major criticism of glass vials is that certain liquids, proteins, and ions can adsorb on to the walls. With Filmware tubes, there is no wall adsorption.
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Packard. A new two-dimensional radioactivity scanner for chromatographic plates and film has been introduced by Packard. The Model 7230 scanner will detect the activity of all β-emitting radionuclides and many low energy, γ-emitting radionuclides on thin-layer plates and film. Chromatograms up to 20 cm × 20 cm are placed on a tray which is inserted into the instrument, and a Geiger detector is arranged to scan the chromatograms in both the x and y directions. A stylus mounted above the detector provides a 'dot' impression on sensitised paper as the detector senses the radioactivity and an exact copy of the distribution of activity is produced as a 'dot' pattern. Many scanning speeds can be selected and collimators to restrict the detection area provide accurate resolution between closely separated regions of activity.
Circle No. 28 on Reader Enquiry Card.

Nuclear Enterprises. NE 1600 from Nuclear Enterprises is a new concept in iodine-125 and cobalt-57 sample counting. By simultaneous counting through-put almost 20 times greater than that of orthodox instruments is achieved.
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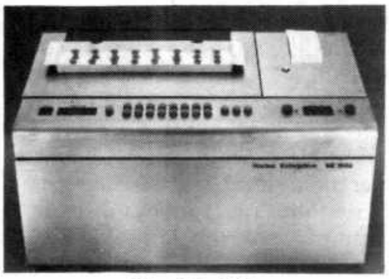
The NE 1600

LKB. The LKB 2258 PMV Cryomicrotome cuts sections of hard materials such as bone or teeth as well as soft tissues or whole animals. It is a compact heavy duty design for stable operation at low temperatures.
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Research Products International Corporation produce the G-M tube environmental monitor, Model 6-70. Supplied with a special compensated MC-70 type G-M tube, this instrument is designed for determining environmental γ radiation levels. The highly sensitive detector is suitable for measuring low intensity radiation within a reasonable time period. Sensitivity is approximately 150 pulses s^{-1} for a $1\,\mu R\,h^{-1}$ exposure rate. Bio-Count is a new cocktail from RPI for counting aqueous samples in a beta counter. It allows the incorporation of up to 3.5 ml of aqueous sample in 10 ml of cocktail without forming a gel. The phase separation region of BioCount, 1.5–1.8 ml, is extremely narrow and in the range that normally does not interfere with most counting applications.
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Amersham Corporation. Several new products and services of interest to users of nuclear instrumentation are available from Amersham. A sample preparation advisory service for liquid scintillation counting is now provided. Should users wish to discuss sample preparation problems, they should call (800)-323 9750 and ask for Technical Services Department. An applications laboratory will perform trials and make recommendations on difficult samples. Amersham has also added a number of new radioactivity standards to their extensive range. The new products include standardised solutions of carbon-14 (as glucose), cerium-139, cobalt-56, gallium-67, indium-111, iridium-192, krypton-85, mercury-197, molybdenum-99, tin-113 and xenon-133. Amersham has an extensive line available of liquid scintillation standards and quenched sets from stock. Custom designed quenched standards sets are also prepared to meet customers' needs.
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Berthold. The Berthold MAG 310 gamma counter is available in two basic versions, the simplest providing output of data in c.p.m. (and for RIA, with nonspecific binding subtraction) and the MAG 310 R, with full multi-user RIA processing on-line. The MAG 310 is a bench mounted microprocessor based instrument, with rack loading for up to 310 samples. Each rack is positively identified numerically and per user. Thus racks out of sequence can be adequately dealt with by the computer software, illuminating a source of weakness in some other systems. In its standard form sufficient shielding is supplied to allow a full range of isotopes to be counted up to and including iron-59. Selection of iodine-125 or another isotope is made automatically via the multi-user system. For laboratories where several operators are to make use of a single system, it is convenient that conversation with the microprocessor (via the output device) is conducted in English.
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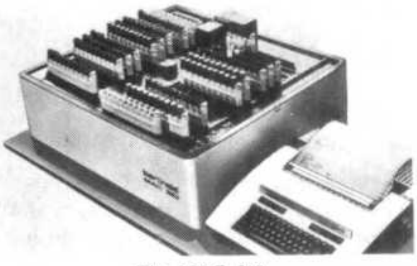
The MAG 310

newly on the market

Liquid nitrogen cryostat. Oxford Instruments. Test trials carried out by Oxford Instruments have shown that their DN700M liquid nitrogen Mossbauer cryostat is capable of operating within a variable temperature range of 77–300 K, with a stability of ± 0.1K. Moreover, it will maintain this degree of stability at 77 K for more than five days. The standard version of the DN700M has a top-loading sample space of 20 mm clear diameter, and a sample change time of only 30 s. The equipment, which incorporates a platinum sensor and a 39 Ω heater, takes only 20 min to cool down and warm up. The tail has a diameter of 63.5 mm with two in-line, wrap-round aluminised mylar windows, giving 25 mm clear access. Nitrogen flow to the heat exchanger is simply controlled using a valve in the gas exhaust line. An activated charcoal sorb is used to maintain a good vacuum.
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Chromatography columns. Wright. A new range of production chromatography columns has been launched by Wright Scientific. Their special features include 'snap-on' sinters and 'pressurised' filling facilities. This large range allows you to choose your exact requirements without buying more than you need.
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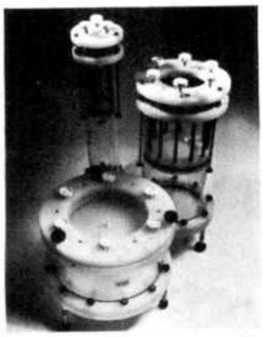

Wright chromatography columns

Light chopper. Rofin. Rofin has developed a variable aperture, variable frequency light chopper which has a chopping blade whose aperture is variable from a very narrow slit to 90°, making the mark-space ratio of the chopped signal continuously variable. Features include chopping frequency variable over 3–200 Hz, high stability of frequency, F/2 input aperture cone, sync output and output control for internal or external frequency drive, 360° phase control and digital LED display housing.
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Integrating sphere calibration source. Glen Creston. The model 420 calibration source is now available from Glen Creston. It consists of an 8 in diameter integrating sphere and a 45 W tungsten–halogen lamp. The lamp is mounted on a movable platform, and an aperture wheel containing five different apertures is mounted in front of the integrating sphere. By altering the lamp position and changing the apertures one can continuously vary the output from the source. The standard source is calibrated for both illuminance and spectral irradiance over the range of 350–1,200 nm. Calibrations for the range 250–350 nm and 1,200–2,500 nm are also available.
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Automatic serum dispenser. Dynatech. An automatic serum dispensing unit, the 'seromat', is now available from Dynatech, and allows up to 60 0.001 or 0.002 ml volumes of typing sera to be dispensed into the corresponding wells of a pre-oiled Teresaki tissue-typing plate. Push-button simplicity of operation means that the complete cycle time for loading a plate, dispensing the serum into the wells and unloading the plate is less than 5s. The seromat dispensing head consists of 60 micropipettes grouped to match the format of a Teresaki plate. Each micropipette has its own 200-μl reservoir and, once the pipettes are primed with a panel of typing sera, up to 200 plates can be seeded in under 30 min.
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Illuminated pocket microscope. Intel Electronic Components announce the availability of a miniature lightweight illuminated microscope which contains a comprehensive graticule enabling the area under inspection to be measured. Calibrations on the graticule are up to 4 mm in increments of 0.1 mm and angles from 30° to 90° are marked, together with a circular measurement of 0.2, 0.3, 0.4, and 0.5 mm. This pocket microscope is only 125 mm long, and has a wide field of view at × 20 magnification. It incorporates a unique built in illumination and easy focusing system. Illumination is powered by standard batteries, 1.5 V, and a screw base 2.3 V 0.2 A bulb.
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Microcellcounter. TOA. A new microcellcounter offering computer capability and an optional data printer has been introduced by TOA Medical Electronics. The Sysmex CC-120 is the nucleus of a modular hematology system. The options which complete the system include: the Sysmex auto diluter AD-220, which aspirates, dilutes and ejects the sample, the ND-900 which displays the sample being analysed, and the DP-411 data printer, which prints a three-digit sample number, three-digit measurement results and three kinds of data. The mercury-free manometer, < 9 s counting time, counting time display, audible aperture clog alarm and reverse aperture flushing are standard features of the CC-120 microcellcounter.
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The Sysmex CC-120

High performance cooling thermostats. KLS. A new range of high performance cooling thermostats have been added to the Julabo equipment available from Kent Laboratory Services, designed for use in research, clinical chemistry and industrial laboratories. The main features of the latest range include proportional temperature control, a virtually noiseless compressor operation, and an automatic on–off switching of compressor to eliminate operating errors. A high capacity circulation pump is built in with a double air ventilation system, and an excess temperature and liquid level protection device is included for unattended day and night operation.
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HPLC column. Whatman. The Whatman Partisil-5 ODS high pressure liquid chromatography column provides a guaranteed minimum of 11,000 plates per column. The column is 25 cm long (4.6 mm diameter) giving a minimum of 45,000 plates per m. This new column is fully silanised, with no remaining adsorption sites, and provides pure C18 reversed-phase chromatography.
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With this Cryo-Microtome you can collect sections ideal for both whole-body autoradiography and tissue sampling. Every section is of the same high quality essential for quantitative distribution studies. The LKB 2250 PMV Cryo-Microtome sections animals up to 150×450 mm easily as well as hard materials such as non-decalcified bone and teeth.

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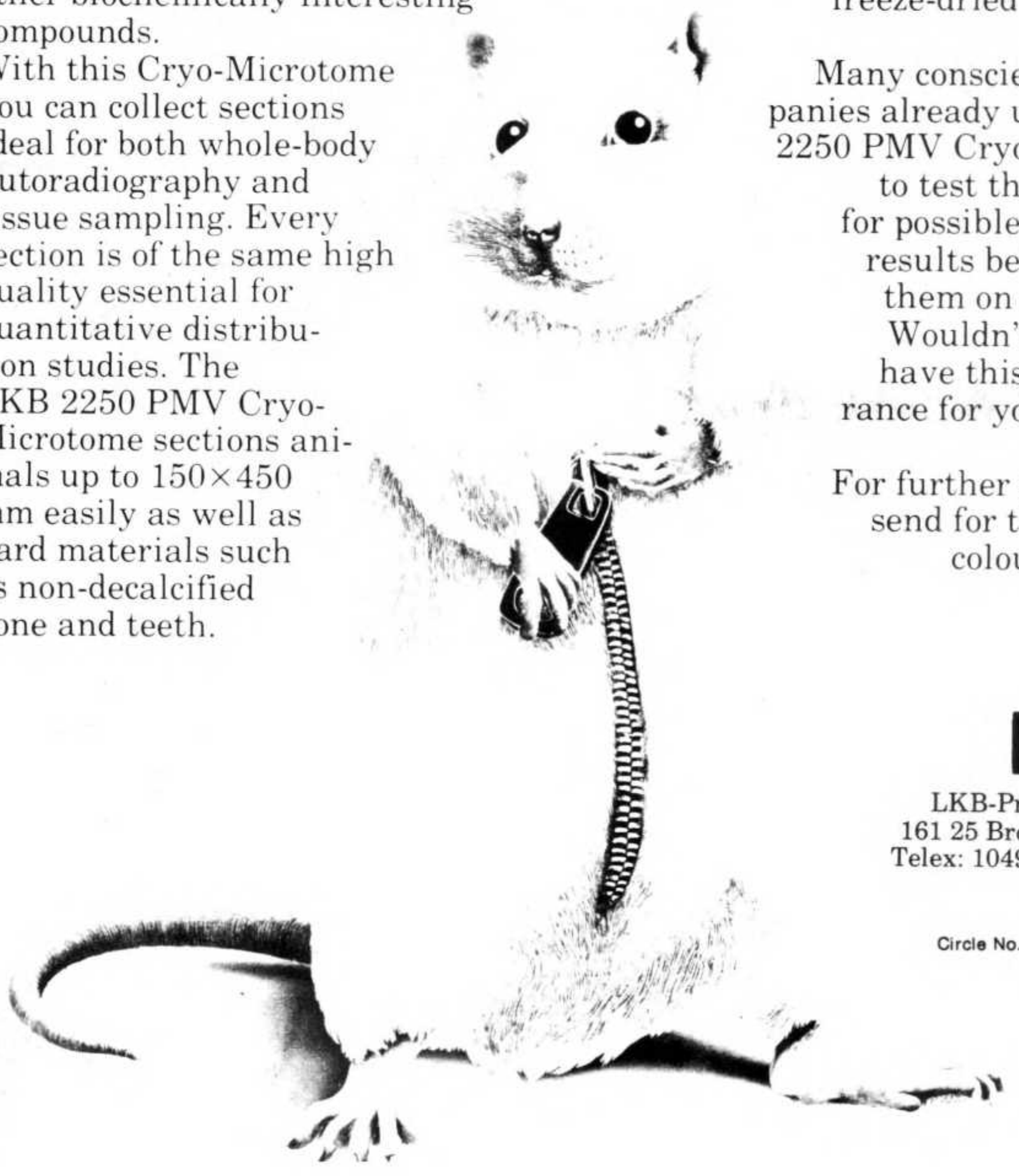
For further information send for the new full-colour brochure.



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Appointment of

Director Dental Unit

(Readvertisement)

The Medical Research Council invites applications for the post of DIRECTOR of a new Dental Unit. The first choice for the location of the new unit is Bristol, but other locations are not excluded.

The research programme of the unit will be a matter for discussion between the new Director and the Council, having regard to the particular interests of the candidate selected.

Candidates should be persons of high scientific standing – preferably, but not necessarily, with a dental qualification – and proven ability in leading a research team.

The salary will be within the range for the Council's Special Appointments Grade, which is equivalent to the Universities' professorial range.

Further particulars, including details of the method of application, may be obtained from Miss N S Jones, 20 Park Crescent, London W1N 4AL.

This post is open to both male and female applicants.

The final date for the receipt of applications is 31 March, 1979.

MRC
Medical Research Council

9145(A)

GRADUATE
or
POSTGRADUATE

in biochemistry, pharmacology, or medicine required for research project on the use of liposome-sequestered cytotoxic agents in experimental chronic inflammatory arthritis. Apply in writing, giving full curriculum vitae to the Administrative Secretary, Strangeways Research Laboratory, Wort's Causeway, Cambridge CB1 4RN. 9189(A)

THE UNIVERSITY OF SUSSEX
SCHOOL OF BIOLOGICAL SCIENCES
RESEARCH OFFICERSHIP

Graduate required to work on a three-year S.R.C. financed research project investigating hormonal effects on the stability of memory traces and of attention. The project is due to commence April 1, 1979, though students graduating in Summer 1979 will be considered.

Starting salary in the range £3,384 to £3,883 p.a. (under review), with superannuation benefits.

Applications as soon as possible to Professor R. J. Andrew, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, from whom further details are available. 9242(A)

PLANT BREEDING INSTITUTE

Maris Lane, Trumpington,
Cambridge CB2 2LQ

HIGHER SCIENTIFIC OFFICER/ SENIOR SCIENTIFIC OFFICER
CYTOGENETICS DEPARTMENT

A Research Worker with experience in plant genetics and cytology is required who is prepared to develop the new techniques of DNA probe analysis in order to promote the understanding of the consequences of particular types of DNA organisation on genetic activities in plants, particularly wheat.

Candidates should have a 1st or Upper 2nd Class Honours Degree in an appropriate biological science and at least two years' postgraduate experience in plant cytogenetics or related research.

The appointment will be as Higher Scientific Officer (£4,101 to £5,448 per annum) or Senior Scientific Officer (£5,154 to £6,898 per annum). At least four years relevant postgraduate experience is required for appointment as Senior Scientific Officer.

Applications with curriculum vitae and the names and addresses of three referees should be sent to the Assistant Secretary (Establishment) by February 12, 1979.

Please quote reference CYT/39.
9195(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BOTANY

Applications are invited for the post of Demonstrator or Senior Demonstrator to assist in teaching and research in bacterial taxonomy. Preference will be given to applicants with experience in numerical methods of classification and identification. The duties of the post will commence on April 1, 1979, or as soon as convenient thereafter; for one year in the first instance, and may be renewed, but not normally more than twice.

The initial salary will be within the range £3,384 to £4,882 per annum.

Applications, together with the names of three referees should be received not later than February 9, 1979, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref. RV/451/N. 9218(A)

PALEOSERVICES LIMITED OF WATFORD

require a

PALYNOLOGIST

with Mesozoic and/or Paleozoic experience to undertake studies of Well sequences and basin analyses.

Salary negotiable.

Please apply in writing to: Paleoservices Limited, Unit 2, Polychrome Building, Sandown Road, Watford, Herts WD2 4UB. 9093(A)

MEDICAL RESEARCH COUNCIL
PNEUMOCONIOSIS UNIT
LLANDOUGH HOSPITAL

PENARTH,
SOUTH GLAMORGAN
CF6 1XW

Applications are invited for a consultant grade post in RESPIRATORY MEDICINE to carry out research into the cause, diagnosis, prevention and treatment of non-infectious extrinsic lung disease. The work includes the direction of a well staffed and equipped respiratory function research laboratory as well as the care of in and out patients at Llandough Hospital.

The successful applicant will be expected to pursue his or her own research as well as collaborate in projects involving other sections of the unit.

Applicants should be qualified to hold a consultant post in respiratory medicine and have special experience in the laboratory investigation of respiratory function. The successful applicant will be offered an unlimited appointment to the Council's clinical staff. Terms of employment will be those normal for M.R.C. Clinical staff with honorary consultant sessions in the National Health Service: Salary £9,528 to £12,084 and compulsory pension scheme.

Applicants should contact the Director, M.R.C. Pneumoconiosis Unit, from whom further information and an application form may be obtained. Closing date—February 24, 1979. 9162(A)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING
CHEMIST/PHYSICIST
(Nutrition Department)

A scientist with considerable technical ability is required to run and maintain an Isotope Mass Spectrometer and an Automatic Optical Emission Spectrometer used in the analysis of ^{15}N. The person appointed will be a member of a small team engaged in studies of nitrogen metabolism in animals.

Applicants should have a degree, H.N.C. or the equivalent in Physics or Chemistry and an interest in biomedical research; or similar qualification in Biochemistry with experience in mass spectrometry. Some knowledge of electronics is desirable.

Appointment will be as Scientific Officer (£2,839 to £4,415) or Higher Scientific Officer (£4,101 to £5,448) according to experience. At least five years' relevant postgraduate research or other approved experience is required for appointment as Higher Scientific Officer.

Application forms are obtainable from the Secretary, N.I.R.D., Shinfield, Reading RG2 9AT. Quote reference 78/36. 9193(A)

GUY'S HOSPITAL MEDICAL SCHOOL
LABORATORY SCIENTIFIC OFFICER

required until September 30, 1980 t work in the Department of Chemica Pathology. Duties will include the *i vitro* cultivation of malaria parasites Previous experience in tissue cultur techniques essential. Salary for qualified Laboratory Scientific Officer no less than £3,261 plus £354 Londo Weighting.

Apply in writing, stating age an giving details of qualifications an experience, to the Secretary, Guy' Hospital Medical School, London Bridge SE1 9RT, quoting Ref. C.H.1.
9228(A)

UNIVERSITY OF GLASGOW
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistant to work with Dr J. P. Goddard in his Scienc Research Council grant-aided projec on structure-function relationships i transfer RNA. The current researc is on the recognition of tRNA^{Ty} and tyrtRNA synthetase from B stearothermophilus.

The position is for one year an salary will be within range 1A of th salary scales for Research and Analogous Staff, £3,883 to £4,382, accordin to qualifications, age and experience Appropriate Superannuation Schem will apply.

Applications enclosing a curriculu vitae and the names of two referee should be submitted by February 9 1979, to Dr J. P. Goddard, Department of Biochemistry, University o Glasgow, Glasgow G12 8QQ.

In reply please quote Ref. No 4346M. 9215(A)

NEUROBIOLOGY AND BEHAVIOUR

A position at the Assistant Professor level is available in the Biology Department, Princeton University. Faculty in the Neurobiology and Behaviour area include M. Constantine-Paton, H. Horn, A. Gelperin, J. Gould, R. Lisk and W. Quinn Evidence of exceptional promise in research is essential Applicants should send curriculum vitae, statement of research experience and three letters of recommendation to Dr Alan Gelperin, Neurobiology Search Committee, Department of Biology, Princeton University, Princeton, N J 08540 Princeton University is an Equal Opportunity/Affirmative Action Employer. 9112(A)

UNIVERSITY OF LEICESTER
DEPARTMENT OF GENETICS
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from persons with an interest in biochemistry or molecular genetics for a postdoctoral research position financed by an M R C. Project Grant. The project will investigate the mechanism of synthesis and assembly into membranes of specific cytoplasmic and outer membrane polypeptides in *E. coli*. This will involve the use of cloned genes for membrane proteins and coupled transcription/translation from DNA templates in *in vitro* systems. The work is likely to involve close collaboration with research groups in the Department studying the physiology of cell division in *E coli* and the cloning of division proteins. The appointment will be tenable for up to 3 years from February 1, 1979 or as soon after as can be arranged

Starting salary £3,660 per annum with full superannuation provision

Applications with names of two referees to Dr I. B Holland, Department of Genetics, University of Leicester, Leicester LE1 7RH

9130(A)

ST. MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Paddington, London W2 1PG
GRADUATE IN PHYSIOLOGY OR PHARMACOLOGY

who will be expected to register for a higher degree, required to join team investigating the mechanism of action to drugs affecting gastric secretion

Appointment, which can be taken up immediately, is for one year initially with prospect of extension for further two years. Project jointly organised between Departments of Physiology and Academic Surgical Unit.

Further information from Mr S Knight (Tel: (01)-723 1252, ext. 133, to whom application with curriculum vitae may be sent at above address

9126(A)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF BACTERIOLOGY
POSTDOCTORAL FELLOW/ RESEARCH ASSOCIATE

required to work on a Cancer Research Campaign sponsored project in tumour immunology. Experience in working with tumours or in the field of cellular immunology would be desirable. Salary on National Range IA.

Send c.v to Dr W. H. McBride, Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland. Please quote Reference 5058.

9090(A)

BRUNEL UNIVERSITY
DEPARTMENT OF APPLIED BIOLOGY

Applications are invited for the post of postdoctoral Research Fellow (for a period of three years) for work on cultured cystic fibrosis cells. The work will be performed in collaboration with Dr Roger Dean, Reader-elect in Applied Biology. The project is being funded by The Cystic Fibrosis Trust Dr Dean's particular interests are in lysosomes, mechanisms of protein degradation, macrophage activation and secretory processes.

The post is particularly suitable for a cell biologist, or a biochemist with experience with cultured cells

Salary within the range £3,910 to £5,367 (under review), plus £450 per annum London Allowance.

Write for application form and further particulars to the Assistant Secretary (Establishment), Brunel University, Uxbridge, Middlesex UB8 3PH, or telephone Uxbridge 37188, extension 49. Closing date February 9, 1979. 9219(A)

UNIVERSITY OF LONDON
Institute of Neurology
DEPARTMENT OF NEUROCHEMISTRY

Applications are invited for two postdoctoral posts Grade 1A in a team working on the metabolism and role of 5-hydroxytryptamine (and related topics) and supported by a recently renewed 5-year M R.C programme grant. Experience in at least one of the following fields essential neurochemistry, neuropharmacology, behaviour Initial salary £4,333 to £6,054 (including London allowance), depending on experience.

Applications with c.v. and names of two referees to Professor G Curzon, Department of Neurochemistry, 33 John's Mews, London WC1, by February 23, 1979

9220(A)

TECHNICIAN/ RESEARCH OFFICER

required to work with Professor Mitchison at Tumour Immunology Unit on viral immunology Work will involve virus preparation and titration, animal work such as immunisations and bleeding, and other immunological techniques Candidates should have an H N C./Degree and at least two years laboratory experience. An interest in immunology and experience in virology and tissue culture would be valuable Salary range £3,261 to £4,902 plus London Weighting

For application form and further information please write or telephone Mrs Nelson, Imperial Cancer Research Fund, Lincoln's Inn Fields, London W C 2. Telephone 01-242 0200. Ext 305 Quoting ref. 77/79 9191(A)

UNIVERSITY OF READING
NATIONAL COLLEGE OF FOOD TECHNOLOGY
Short-term Lectureship in Applied Biochemistry

Applications are invited for the above post tenable at the National College of Food Technology, Weybridge, Surrey, for the duration of the Summer Term (mid-April—beginning July, 1979) The duties are to give a short course of lectures on food biochemistry and to supervise related practical classes Payment for the Term will be within the range £1,200 to £1,600.

Applications with a curriculum vitae should reach the Principal, National College of Food Technology, St George's Avenue, Weybridge, Surrey by January 31, 1979. 9192(A)

CSIRO AUSTRALIA

Aircraft Operations Manager

Division of Cloud Physics
Sydney, NSW

CSIRO has a broad charter for research into primary and secondary industry areas The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

General: The Divisions' research activities are associated with the natural mechanisms of cloud and rain formation, artificial induction of rainfall by techniques such as cloud-seeding, and studies of atmospheric particles

Duties: Responsible for the co-ordination and performance of activities associated with the Division's use of aircraft

Advise research staff concerning the installation of scientific equipment in aircraft, undertake the layout design of such installations to meet research requirements and Department of Transport standards and liaise with installation contractors.

The appointee may be required to provide advice about aircraft available for charter and their suitability for research purposes, to negotiate with charter companies and to be generally responsible for the modification and use of chartered aircraft. The appointee will also be required to act as "Field Manager" during the course of a major cloud seeding experiment to be concucted over a five year period in Western Victoria As Field Manager the appointee will be responsible for all aspects of aircraft operations associated with the experiment, which involves the use of three aircraft. It will therefore be necessary for the appointee to be located at *Mildura* from August to mid November commencing in 1979 and over the same period for the ensuing four years.

Qualifications: An appropriate degree or diploma, or equivalent qualification Applicants should preferably have had considerable experience in the aircraft industry and be familiar with the operation, maintenance and modification of a wide variety of aircraft A good knowledge is required of D.O T airworthiness standards, especially those concerning structural modification and equipment installation.

Applicants should be physically fit to fly and prepared to participate in field operations involving absences from Sydney

Salary: Within the range $A17,115 to $A20,867 p.a Appropriate allowances are payable for extended duty away from Sydney.

Tenure: Indefinite with superannuation.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 425/59 should reach

The Personnel Officer, Australian Scientific Liaison Office,
Canberra House, 10-16 Maltravers Street, London WC2R 3EH,
by 16th FEBRUARY 1979.

Applications in U.S A and Canada should be sent to:
The Counsellor Scientific, Embassy of Australia,
1601 Massachusetts Avenue, N.W , Washington, D.C.,
20036, U.S.A. 9208(A)

AN FORAS TALUNTAIS
LAND RECLAMATION RESEARCH

Applications are invited for a post in an expanding land reclamation research programme, with particular emphasis on drainage The post will be in the Western Research Centre, at Creagh Station, Ballinrobe, Co. Mayo

ESSENTIAL: Honours University Degree, or equivalent, in Engineering, Agricultural Engineering or Agricultural Science

DESIRABLE: Postgraduate study and/or experience in soil/water relationships as affecting land reclamation and use.

Appointment up to and including Senior Research Officer level (salary maximum £8,465) depending on qualifications and experience Marriage and Childrens' allowances. Non-contributory Pension Scheme and provision for Widows' and Orphans'.

Application form and further particulars from Personnel Department, An Foras Taluntais, Headquarters, 19 Sandymount Avenue, Dublin 4. (Tel 688188). Latest date for receipt of completed forms Wednesday, January 24, 1979.

9158(A)

Biochemist

The Plant Protection Division of ICI wishes to recruit a biochemist with postgraduate research experience to play a part in the discovery of new insecticides An understanding of neurobiology would be desirable, but practical experience of insect biochemistry is not essential However, candidates must have considerable scientific flair and imagination, and the ability to draw on research in other fields

The successful applicant will join a multidisciplinary project team and will be responsible for the work of two graduate biochemists The job holder will be required to initiate research in areas of insect biochemistry that could lead to a synthetic programme or produce *in vitro* methods for detecting active chemicals Complementary work will be concerned with evaluating the mode of action of promising new insecticides and gaining an early understanding of the factors that may contribute to the development of resistance

We offer competitive salaries and there is a Company Profit Sharing Scheme Assistance with removal expenses and house purchase may also be available The Research Station is situated on a pleasant farm estate between Bracknell and Maidenhead in Berkshire .

If you are interested in this vacancy please write or telephone for an application form to

Mr D Spence, Personnel Officer, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berkshire Telephone Bracknell 24701, ext 3350.

Please quote reference 79/Biochem

ICI

Plant Protection Division

9177(A)

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE

Head of the

Microbiology Division

Senior Principal Scientific Officer

Applications are invited for this post from scientists with a broad experience in Microbiology.

Distinction in a relevant microbiological research area, preferably with a bacteriological background, is required and experience in food-related areas will be of special value. The total staff of the Division will be approximately 25 with at least five Principal Scientific Officers and appropriate support staff.

The Institute is housed in excellent laboratories in Norwich and takes a major role in the Council's research effort relating to food in the U.K.

The salary range for this appointment is £10,300 × 2 to £11,300 and there is a non-contributory superannuation scheme.

Further particulars and application forms are available from the **Secretary, ARC Food Research Institute, Colney Lane, Norwich, NR4 7UA, quoting Ref: 77/10. Closing date February 5, 1979.** 9197(A)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

(University of Reading)

UTILISATION OF SKIMMED MILK

The following temporary research staff are required to work on a project supported by the E E C and concerned with improved utilisation of skimmed milk:

Two protein chemists/enzymologists,

A food technologist or chemical engineer;

A protein chemist, physical chemist or bio-physicist

Applicants should possess a 1st or upper 2nd class Honours Degree and have had at least four years' post-graduate research or other relevant experience Salaries will correspond to those of the Scientific Civil Service

Application forms are available from the Secretary, N I R D, Shinfield, Reading RG2 9AT Please quote reference 78/45 9194(A)

University of Cambridge LECTURESHIP Applications are invited for a University Lecturer in Botany to take up appointment from October 1, 1979 or as soon as possible thereafter The person appointed will be expected to provide teaching in the field of whole plant physiology and/or ecological plant physiology and to have research interests in the same areas of plant physiology The appointment will be for three years in the first instance, with the possibility of reappointment to the retiring age The salary is on an incremental scale rising from £5,367 to £8,257 per annum Applications with the names of two referees should be sent by March 31, 1979 to the Secretary of the Faculty Board of Biology 'A' Appointments Committee, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX, from whom further particulars may be obtained 9226(A)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF ZOOLOGY

RESEARCH ASSISTANT

Applications are invited from graduates in zoology and those intending to graduate in 1979 to work with Dr D F Houlihan on "Energy cost and mechanisms of Transepithelial Transport in Insects"

Appointment will be from April 1, 1979, or as soon thereafter as possible, and will be for three years Commencing salary within Range 1B, £3,384 to £3,883 per annum, with appropriate placing

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by February 9, 1979, quoting Reference DH2 9163(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of Technician to assist with research in insect biochemistry Minimum qualification for this post is an O N C, but applications will be welcomed from candidates holding H N C or graduates or a degree in Biochemistry or Zoology Some experience of insect physiology an advantage but not essential The post is tenable for 2½ years in the first instance with a possibility of renewal Salary within a range up to £2,835 per annum (under review) according to qualifications and experience

Application forms may be obtained from The Registrar, The University, P O Box 147, Liverpool L69 3BX Quote Ref RV/456/N

9217(A)

UNIVERSITY OF SURREY

CHEMISTRY DEPARTMENT

TECHNICIANS

TECHNICIAN GRADE 5

£3,186 to £3,720 (under review)

A Grade 5 laboratory technician i required to assist in the daily runnin of laboratories with special responsibility for the departmental radiochemical laboratories The routine and research procedures may include operation and maintenance of a variety o sophisticated instruments, assistin with undergraduate or postgraduat courses, radiosynthesis, and maintenance of safety standards

Qualifications sought, H N C., H N D /Advanced City & Guilds and 8 to 9 years' experience.

TECHNICIAN GRADE 4

£2,955 to £3,402 (under review)

A Grade 4 Technician is required to assist in the running of the undergraduate laboratories, with specia responsibility for the Physics laboratory within the Chemistry department The Physics laboratory is an integra part of the Physical Science degree course and therefore a background in physics is desirable in addition to chemistry.

Qualifications sought O N C, O N D /Ordinary City & Guilds/2 A Levels and 5 to 8 years' experience.

Application forms for both of these posts may be obtained from the Staff Officer, University of Surrey, Guildford, Surrey GU2 5XH, or telephone Guildford 71281, extension 452

Closing date for applications February 12, 1979 9205(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF PHYSICS

TWO LECTURESHIPS

Applications are invited for two lectureships in the Department of Physics. The successful candidates will be required to contribute fully to the teaching programme in the Department One of them will be expected to join the Nuclear Structure Research Group, the other to join one of the other research groups in the Department They will be expected to take up their appointments not later than October 1, 1979.

Salary will be on the scale £3,883 to £7,754 (under review) plus superannuation

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, P O Box 363, Birmingham B15 2TT, to whom applications (three copies), including full curriculum vitae and the names of three referees should be sent by Friday, February 2, 1979 Please quote ref. ND1. 9222(A)

MEDICAL RESEARCH COUNCIL

Clinical Oncology and Radiotherapeutics Unit

require

a biochemical pharmacologist to participate in a programme on the pharmacokinetics and the metabolism of drugs and radiation sensitisers in animals and man Experience of high performance liquid chromatography is essential

The successful candidate will conduct routine drug estimations and will take part in the Unit's on-going research programme

The appointment will be for five years with a possibility of eventual tenure to retiring age. Candidates must have at least an upper second class honours degree and six years' relevant postgraduate experience

Salary on scale starting from £5,367 per annum

Applications, with full c v and names of two referees, should be sent to Miss F M Taylor, M R C Clinical Oncology and Radiotherapeutics Unit, The Medical School, Hill Road, Cambridge CB2 2QH, to be received not later than February 12, 1979.

9225(A)

UNIVERSITY OF WALES

UNIVERSITY COLLEGE OF SWANSEA

Senior Research Assistant

Applications are invited for the vacancy of

Senior Research Assistant in the Department of Botany and Microbiology.

The post is financed by N.E.R.C., to support work, under the supervision of Professor P. J. Syrett, on the biochemistry and physiology of nitrogen assimilation by marine photoplankton. Applicants should have a Ph.D. degree and research experience in biochemistry.

The appointment, which will be for one year in the first instance, with the possibility of renewal for a further two years, will be on the scale up to £4,382 per annum, together with U.S.S./U.S.D.P.S. benefits.

Further particulars and application forms (two copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by Friday, February 2, 1979. 9161(A)

AUSTRALIAN NATIONAL UNIVERSITY OFFICE FOR RESEARCH IN ACADEMIC METHODS

An academic position is vacant in the Office for Research in Academic Methods (Head Mr A. H. Miller). O.R.A.M. was established in 1975 to improve facilities for teaching and learning within the University, to collect data necessary for educational planning, and to make the findings of educational research available to teaching and administrative staff.

Applicants should have experience in university teaching and should be qualified to undertake research into one or more aspects of higher education as part of a small independent team. There may be opportunities for a small amount of teaching in the appointee's own discipline, as this will help maintain contact with students and will provide the chance to practise innovations in course design, teaching and assessment. The appointee may be asked from time to time to be Acting Head of the Office in the absence of the Head.

Appointment will be for three years in the first instance, with the possibility, following review of reappointment for a further fixed term or to age 65. A three year secondment from an existing tenured position could be negotiated.

This post was advertised earlier as Research Fellow or Senior Research Fellow. The terms have now been slightly varied and the title has not yet been resolved. The applications received for the post when it was last advertised will be considered, unless candidates indicate that they are no longer interested.

Commencing salary will be in accordance with qualifications within the ranges $A15,179 to $A19,940 per annum or $A20,365 to $A23,737 per annum. Current exchange rates $A1 58p $US1.14.

Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain the further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. The closing date for receipt of applications is *February 16, 1979*. 9154(A)

Scientists for Neutron Beam Research

Rutherford Laboratory

The Rutherford Laboratory is constructing a new neutron source, known as the Spallation Neutron Source (SNS) which will provide intense pulsed neutron beams for condensed matter research by scientists from universities and polytechnics.

This is a major project of world significance which is planned for initial operation by 1982.

Preparation for exploitation has begun and this will require the progressive recruitment of neutron scattering specialists during the next few years to contribute in various areas, notably the design of neutron scattering instruments for the SNS, supervision of their operation, and the planning of support facilities such as sample preparation laboratories. In the short term, development studies on pulsed source instrumentation will be made using the Harwell electron linac and pulsed sources elsewhere. In addition the successful applicants will be expected to participate in scientific research projects usually in collaboration with the user community, and using the facilities at Harwell and the Institut Laue-Langevin (ILL), Grenoble. It is the intention that some of those recruited will be invited to work for extended periods at ILL to broaden their experience and to contribute to the ILL programme. There are opportunities for suitably qualified scientists, including theorists and experimenters, both for permanent appointments and for secondment. Applications will be considered from those able to fill posts promptly as well as later during 1979.

In the case of secondment, the Rutherford Laboratory would welcome applications or enquiries from staff in universities or similar institutions who have considerable experience in neutron scattering and who would be interested in working at the Laboratory for periods of 2-5 years. The precise terms of secondment would be subject to negotiation between a successful candidate, his employer and the Laboratory.

Appointments to permanent posts in the Science Group will be made at SO/HSO levels. Applicants for these posts will preferably have a good honours/PhD degree plus relevant experience. Exceptionally an appointment might be made at a more senior level (SSO or PSO).

Salary scales: Scientific Officer £3,037 to £4,724.
Higher Scientific Officer £4,388 to £5,829.
Contact:- Jane Griffiths in the Personnel Group on Abingdon (0235) 21900 Ext. 510. Or write to her quoting reference VN 763.

Closing date for applications February 9th 1979.

RUTHERFORD
Science Research Council
Rutherford Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX. Tel Abingdon 21900

9199(A)

CSIRO AUSTRALIA

Division of Fisheries and Oceanography
Sydney, NSW

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: FISHERIES BIOLOGY

General: The Division is Australia's principal marine research institution with about 70 scientists investigating the physical, chemical and biological features including fisheries of the oceans around Australia. Headquarters of the Division are at Cronulla, Sydney, with regional laboratories in Perth, Brisbane and a field laboratory at Karumba. On-line access to a Cyber 76 computer is available. Two well-equipped medium sized chartered vessels are available for oceanographical research and there are several smaller CSIRO vessels.

1. Research Scientist

Reference Number 320/611

Duties: To take a leading role in the development and execution of a research programme on marine fish resources around Australia including the supervision and co-ordination of other researchers in this field. Initially attention will be directed to demersal and pelagic resources associated with the continental mass. Field investigations on the Division's vessel and on fishing vessels in the open sea will be required. Temporary posting to other parts of Australia might be necessary.

Qualifications: A biologist with considerable experience is being sought. Basic formal qualifications are Ph.D degree or equivalent qualifications in one of the ecological sciences or fisheries with training in mathematics and statistics, together with demonstrable research ability, and ability to work at sea.

Salary: Senior Research Scientist: $A18,819 to $A21,543 p.a. or Principal Research Scientist: $A22,353 to $A25,354 p.a.

Tenure: Indefinite with superannuation.

2. Postdoctoral Research Fellow

Reference Number 320/610

Duties: To participate in the development and execution of a research programme on marine fish resources around Australia. Initially attention will be directed to demersal and pelagic resources associated with the contental mass. Field investigations on the Division's vessels and on fishing vessels in the open sea will be involved. Temporary posting to other parts of Australia may be required.

Qualifications: A Ph.D degree or equivalent in one of the ecological sciences or fisheries with training in mathematics and statistics, together with demonstrable research ability, and ability to work at sea.

Salary: Research Scientist: $A14,829 to $A18,177 p.a. or Senior Research Scientist: $A18,819 to $A21,543 p.a.

Tenure: 3 to 5 year fixed term appointment with superannuation.

Applications IN DUPLICATE, stating FULL personal and professional details, the names and addresses of at least two professional referees, and QUOTING THE APPROPRIATE REFERENCE NUMBER(S) should reach:

The Personnel Officer, Australian Scientific Liaison Office,
Canberra House, Maltravers Street, London WC2R 3EH
by 16th FEBRUARY 1979

Applications in U.S.A. and Canada should be sent to:
The Counsellor (Scientific) Embassy of Australia,
1601 Massachusetts Avenue N.W., Washington, D.C. 20036

9212(A)

AHMADU BELLO UNIVERSITY, NIGERIA

Applications are invited for the posts of SENIOR LECTURERS and LECTURERS in the DEPARTMENT OF HUMAN ANATOMY. For the post of Senior Lecturers, candidates should possess a higher degree, have considerable teaching and some research experience preferably at university level. For the post of Lecturers, candidates should possess a first degree with considerable years teaching experience since qualification. All appointees will be expected to assist with departmental administration as and when required and also provide a service to the community whenever possible. Salary scales: Senior Lecturer N7,764 to N8,724 per annum (£6,161 to £6,923 per annum sterling). Lecturer I N6,444 to N6,984 per annum (£5,114 to £5,542 per annum sterling). (£1 sterling equals N1.26). Fringe benefits include pension scheme or contract addition of salary (partly taxable). There may be salary supplementation of salary for Senior Lecturers of £2,892 per annum (sterling) for married appointees and £726 per annum (sterling) for single appointees (presently under review and free from all tax) and provision of children's education allowances and holiday visit passages. Lectureships will not attract the supplement. The appointment may be either on fixed term normally of two years contract renewable by mutual agreement or till retiring age of sixty. Family passages, biennial overseas leave, car allowance and pension scheme, partly furnished accommodation at low rental. Detailed applications (two copies) with curriculum vitae and naming three referees should be sent direct to the Registrar, Ahmadu Bello University, Zaria, Nigeria, by February 27, 1979. Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 9184(A)

THE UNIVERSITY OF LIVERPOOL
CYTOGENETICS UNIT
SCIENTIFIC OFFICER (CYTOGENETICIST)

Applications are invited from Science graduates for a basic grade post on the Whitley Council "A" scales in this unit which provides the Cytogenetics Service for the Mersey Regional Health Authority.

Experience in human cytogenetic techniques/tissue culture is desirable.

Salary within a range £3,486 to £4,275 per annum depending on qualifications and experience. Applications in writing together with two references should be made as soon as possible and no later than Friday, February 9, to The Registrar, The University, P.O. Box 147, Liverpool L69 3BX.

Quote Ref: RV/446/N.

9156(A)

THE UNIVERSITY OF MANCHESTER
EXPERIMENTAL OFFICER
DEPARTMENT OF PHARMACY

Applications are invited from graduates in chemistry, biochemistry or a related science. The successful candidate will develop methods for determination of drugs in biological fluids, using modern chromatographic and radioactivity techniques, help plan and run kinetic experiments in animals and man, and help in processing the data using computational techniques. Salary range £3,384 to £5,604 per annum. Superannuation under U.S.S. Particulars and application forms (returnable by January 29) from the Registrar, The University, Manchester M13 9PL. Quote ref: 1/79/N.

9160(A)

EUKARYOTIC MOLECULA BIOLOGY
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF IOWA

The department invites applicatio and nominations for a faculty po tion at the Assistant Professor Associate Professor level that w become available July 1, 197 Responsibilities will include particip tion in the department's medica graduate, and undergraduate teachi programmes as well as in independe research.

Applicants should hold a doctor degree and have postdoctoral expe ence and/or previous faculty expe ence. Candidates will be evaluated the basis of their dedication to exc lence in teaching and research and their capacities to develop a vigoro research programme in eukaryot molecular biology.

Applicants should send curriculu vitae, list of publications, a summa of research interests, and have thr letters of recommendation submitt on their behalf to:

Dr Thomas W. Conway,
Chairman,
Faculty Search Committee,
Department of Biochemistry,
University of Iowa,
College of Medicine,
Iowa City, IA 52242.

Deadline for applications a nominations: March 1, 1979.

The University of Iowa is an Equ Opportunity and Affirmative Acti Employer. 9170(A)

INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTUR
Ibadan, Nigeria
Position:
ENTOMOLOGIST—CEREA CROPS

Entomologist sought with Ph. and at least three years' resear experience in food crops, preferab cereals, to work as member of mul disciplinary team responsible for i provement of rice and maize. Pla host resistance work preferred.

Salary and benefits at internation level commensurate with qualific tions. Two-year renewable contra Position available immediately.

Applications should be sent wi bio-data and three references to:

Director General,
International Institute of Tropic
Agriculture,
Oyo Road,
PMB 5320,
Ibadan, Nigeria. 9167(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND
FACULTY OF MEDICINE
ASSISTANT PROFESSOR OF IMMUNOLOGY

Immunologist/Immunogeneticist quired at Assistant Professor (a Postdoctoral fellow) level to j others in a medical school immunolo group. Special research strengths this group are a good HLA (International workshop standar complement allotyping, collaborati relationships with the blood bank, b chemical genetics, cytogenetics a with clinical investigators, plus availability of large families. group is also involved in teaching, a clinical immunology diagnos laboratory and with a transplantati programme.

For more information please wri preferably sending detailed curricul vitae plus any relevant reprints, to W. H. Marshall, Professor of munology, Faculty of Medici Memorial University of Newfoundla Canada, A1B 3V6. 9137(A

CURRAUN FISHERIES LTD.

a company within the Guinness Group

require a

MANAGING DIRECTOR

Qualifications: Biologist, with expertise in the farming of salmon and rainbow trout.

Location: Newport, Co. Mayo, Ireland.

Salary: According to age and experience.

Assistance with relocation expenses.

Following research, Curraun Fisheries Ltd., is about to build a hatchery and seacages in Co. Mayo. A Managing Director is required to lead and augment an existing small team and develop the enterprise, including, in due course, further such operations.

Applicants should be commercially minded, with initiative and drive, to provide the growth envisaged by the Company Future prospects are good for the individual who can achieve the Company's targets

Reply, with appropriate career and other details to:

C. T. Howlett,
St. James's Gate,
Dublin 8.

9206(A)

UNIVERSITY OF SOUTHAMPTON

SHIP SCIENCE

Vacancies for a

MATHEMATICIAN/ PHYSICIST

and a

NAVAL ARCHITECT

Applications are invited to fill two posts to continue research into the capsizing of ships This work is being sponsored by the Ministry of Defence for a further period of three years

The first appointment, to take effect immediately, is for a postdoctoral Research Fellow who should be an Applied Mathematician or Theoretical Physicist with experience in hydrodynamics An additional knowledge of the mechanics of non-linear systems would be advantageous

The second appointment, to take effect after July 1, 1979, is for a Research Assistant who should have a good degree in Naval Architecture with a knowledge of ship dynamics and hydrodynamics

Salaries will depend on age and experience but will be in a range up to £4,882 for the Fellowship and £3,882 for the Assistantship U S S superannuation benefits apply

Further details may be obtained from D A S Copland, The University, Southampton SO9 5NH to whom applications giving a curriculum vitae and the names of two referees should be sent not later than February 12 Please quote reference No 181/R/Na. 9148(A)

UNIVERSITY OF MELBOURNE

Australia

RESEARCH FELLOW GRADE II (LIMITED TENURE) IN PHYSICAL OCEANOGRAPHY OF THE SOUTHERN OCEAN

in the

DEPARTMENTS OF MATHEMATICS AND METEOROLOGY

A position has been created, in conjunction with a New Development Programme for Antarctic Studies and Resources, for a Research Fellow to work jointly in the Mathematics and Meteorology Departments of this University primarily on the development of a model of the Southern Ocean circulation with some emphasis on sea/air/ice interactions.

The candidate should preferably have a background in physical oceanography and some knowledge of numerical modelling. Applicants should also state any field experience they may have

SALARY $A15,179 to $A19,940 per annum.

Further information, including details of application procedure and conditions of appointment, is available from the Registrar, University of Melbourne, Parkville, Victoria, 3052, Australia or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications close on *March 15, 1979.* 9179(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

THE JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

RESEARCH FELLOWS

(Several positions)

Applications are invited from candidates who hold a Ph D degree or who hold medical, dental or veterinary qualifications and have had some research experience.

The Departments in the School and their fields of research are.

Biochemistry (Head, Professor F. Gibson, F.A.A., F.R.S.): Biochemical and genetic studies on bacterial membranes, oxidative phosphorylation, active transport, NADH dehydrogenase, ubiquinone biosynthesis Kinetic mechanism and regulation of enzymes concerned in aromatic biosynthesis.

Clinical Science (Acting Head, Dr M A Denborough). Biochemical and pharmacological studies on membrane function in relation to human disease Emphasis at present is on calcium, cyclic nucleotide and prostaglandin metabolism, asthma, diseases of muscle, malignant hyperpyrexia, platelet function, atherosclerosis, thrombosis and haemostasis, osteogenesis imperfecta, clinical pharmacology and clinical immunology

Experimental Pathology (Acting Head, Dr W. J Cliff): Research in this Department, which traditionally combines morphological and biochemical studies, has been on *cardiovascular disease*, in terms of the earliest occlusive lesions, endothelial permeability and experimental arterial grafting; topography, differentiation and regression of vascular beds, *neoplasia*, in terms of cyclic nucleotide metabolism and levels in body fluids for cancer diagnosis, and the effects of ionizing radiation on mammalian tissues, and *degenerative diseases* with the search for a hormone for fibrous tissue contraction with the induction and treatment of arthritis and with the study of both local and systemic changes associated with inflammation; the mechanisms of cellular and subcellular motility, using cinephotomicrographic, fine structural, cytochemical and biochemical techniques.

Human Biology (Head, Dr R L. Kirk): Human population genetics; disease susceptibility and red cell metabolism and function.

Immunology (Head, Professor B. Morris, F.A.A.): Genetic basis of disease resistance in economic livestock, physiological studies of the immune response, transplantation biology, immunobiology of cancer, tolerance, foetal-maternal interactions, physiology of the lymphoid apparatus, immunological studies of cattle chimeras.

Medical Chemistry Group (Head, Dr D J. Brown): The Group studies the synthesis, properties, stereochemistry, and biological activity of heterocycles (carbocyclic molecules including nitrogen or another foreign atom) related to natural pyrimidines, pteridines, or purines; the equilibria involving metal ions and cell constituents such as amino acids or proteins, and the spectroscopic properties of simple molecules Attention is being given to the therapeutic applications of such research.

Microbiology (Head, Professor G. L. Ada, F A.A.): Reactions of antigens with lymphoid cells, particularly T cell recognition of membrane antigens, structure, synthesis and function of viral nucleic acids; tissue cultural studies of the growth and genetics of pox, polio and other viruses; immune defence mechanisms against viruses, bacteria and tumours, immunogenetics, the chemistry, antigenic properties and epidemiology of influenza virus, epidemiology and adaptation of arboviruses; activities of lymphocyte sub-populations and responses to modified antigens, properties and roles of histocompatibility antigens.

Parmacology (Head, Professor D R. Curtis, F.A.A., F.R.S.): Neuropharmacology of central synaptic transmission, including that of central pain pathways, neurochemistry of central amino acid receptors.

Physical Biochemistry (Head, Professor L. W Nichol). Physical and chemical characterisation of biological macromolecules, interacting protein systems including studies on subunit assembly, self-associations and acceptor-ligand binding, amino acid sequence of lactoferrin; comparative studies of protein denaturation and hydration, physical and kinetic studies on sulphatases and related enzymes; molecular conformation and structure-activity relations of drugs and neurotransmitters, enzyme transition state analogues.

Physiology (Head, Professor P. O Bishop, F.A.A., F R.S.); Sensory neurophysiology especially in relation to vision and visual optics, neurohistology and the biophysics to muscular contraction.

Appointments normally will be for two years but in exceptional circumstances may be for three years and extended to a total period of five years

It is hoped that the successful candidates will be able to take up their appointment in the first half of 1979.

APPLICANTS SHOULD ARRANGE FOR REPORTS FROM THREE ACADEMIC REFEREES TO BE SENT TO THE UNDERSIGNED IN SUPPORT OF THEIR APPLICATION.

CLOSING DATE· *FEBRUARY 15 1979.*

Commencing salary will be in accordance with qualifications within the range $A15,179 to $A19,813 per annum Current exchange rates $A1 58NP $US1 14.

A salary loading of up to $A5,000 per annum may be paid to an applicant holding appropriate medical qualifications.

OTHER CONDITIONS

Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute Assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time

Further information may be obtained from the Heads of the respective Departments.

P O Box 4,
Canberra, ACT 2600,
Australia

G. E. Dicker
Academic Registrar

9149(A)

free university amsterdam

The **Faculty of Medicine** has a vacancy for a

READER ("Lector") in Histology.

Applicants should have practical teaching experience in at least a part of histology as well as research experience, preferably in the histofysiology of the immune response

The Free University was founded in 1880 as a private institution and is now a fully state subsidized private University

The preamble to its charter speaks of the Christian idea of service as the university's guiding principle.

It would be appreciated if candidates would express their attitude towards this principle in their application

Non-Netherlands applicants are expected to acquire a reasonable command of the Dutch language within about two years

Suggestions concerning qualified candidates for this function should be submitted to the chairman of the nomination committee, Prof Dr A H M Lohman, (Van der Boechorststraat 7, 1081 BT Amsterdam, tel 020 - 548 27 23), who is also willing to give further information

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Applications, accompanied by a curriculum vitae, a list of publications and three names of persons, acquainted with the applicant's academic and professional standards, should be sent within four weeks after appearance of this announcement to Dienst Personeelszaken (Personnel Department), Vrije Universiteit, De Boelelaan 1105, postbox 7161, 1007 MC Amsterdam, quoting reference number 219 - 2628

9240(A)

VU

Wellcome

Biochemist/Enzymologist

Beckenham, Kent

Wellcome Reagents Ltd—a rapidly growing part of the international, British-owned Wellcome Foundation Ltd.—researches, manufactures and markets biological diagnostic products for medical and veterinary laboratory use world-wide.

We seek a scientist to join a small team working on enzyme-based immunoassay systems for the analysis of clinical samples in the Research and Development section of Wellcome Reagents Ltd.

Candidates should have some postgraduate experience including work in the field of enzymology (particularly purification and assay) and should be prepared to contribute actively to the development of novel diagnostic systems.

Salary offered will depend on qualifications and ability but is likely to be around £5000 initially, within the range £3651 to £5522 or £4997 to £7859. There is considerable scope for job development as the organisation continues to grow. The post is based at The Wellcome Research Laboratories, situated in pleasant parkland surroundings, within easy reach of Bromley and Beckenham, about 12 miles from Charing Cross. Conditions of employment are attractive and include 4 weeks' holiday, company pension fund, sick pay scheme, subsidised canteen and assistance with relocation expenses, where appropriate.

Please write, giving details of relevant experience and qualifications, to
R V Sutton, Personnel Officer,
The Wellcome Research Laboratories,
Langley Court, Beckenham, Kent BR3 3BS,
quoting reference number U.978.

9223(A)

THE UNIVERSITY O SYDNEY

LECTURESHIP IN BIOCHEMISTRY

To start as soon as possible I
will consist of teaching and re
within the Department An inter
protein biochemistry would be
advantage.
The position may be filled
on a tenured basis or, in the
instance, for a probationary peri
three years with the possibilit
tenure being given during or s
after that period
Salary range $A15,786 to $A
per annum.
Applications including curric
vitae, list of publications and
of three referees by *February 5,*
to the Registrar, University of Sy
NSW 2006, Australia, from
further information available Inf
tion also available from Asoc
of Commonwealth Universities (A
36 Gordon Square, London W
0PF. 9150

UNIVERSITY OF MALA

FACULTY OF SCIENCE

Applications are invited for
posts of *LECTURER IN*
DEPARTMENT OF GENETIC
CELLULAR BIOLOGY in the
of VIROLOGY/BACTERIOL
Candidates should possess a good
degree in Microbiology and
teaching and research experienc
the required field They should
possess at least a Master's degr
a Ph.D in the required field.
Salary Scales (All incl
[Approx. stg equiv] £3,602 by
to £3,939/£4,107 by £168 to £4
£4,780 by £280 to £6,182/R
Point £6,462 by £336 to £6,799
commencing salary for Lecturers
a Ph D degree shall be £4.107
annum
Further particulars and appli
forms are obtainable from the
ciation of Commonwealth Unive
(Appts), 36 Gordon Square, L
WC1H 0PF.
The closing date for the recei
applications is *February 6, 1979*
9152

OIL POLLUTION

An international organisation in the field of oil pollution is seeking a person to be based in their London office to assist the existing technical staff in their in-depth studies of oil spill incidents, clean-up techniques and effects Applicants should be aged 25 to 35 and should possess a degree or equivalent qualification in a relevant subject. Experience in oil pollution or a related field is desirable but not essential Salary negotiable depending upon experience and qualifications

Applications, which should include a curriculum vitae, should be sent by Febaruary 12 to:

The Technical Manager,
International Tanker Owners'
Pollution Federation Limited,
Plantation House,
31-35 Fenchurch Street,
London EC3. 9165(A)

UNIVERSITY OF ABERDEEN
DEPARTMENT OF ZOOLOGY
RESEARCH ASSISTANT

Applications are invited from graduates in Zoology and those intending to graduate in 1979 to work with Dr D F Houlihan on the Development of Insect Flight Muscle Experience with electron microscopy would be an advantage

Appointment will be from April 1, 1979, or as soon thereafter as possible, and will be for one year Commencing salary within Range 1B, £3,384 to £3,883 per annum, with appropriate placing

Further particulars from The Secretary, The University, Aberdeen with whom applications (two copies) should be lodged by February 9, 1979, quoting reference DH1 9164(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL
DEPARTMENT OF HISTOPATHOLOGY

Graduate Neuroscientist required, with experience in morphological methods, to investigate the effects of tobacco alkaloids on brain development Some biochemical experience desirable The appointment will be for one year in the first instance

Salary up to £4,795, dependent on experience and qualifications

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 6/245/N
9151(A)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
(University of London)
Keppel Street, WC1E 7HT

DEPARTMENT OF MEDICAL PROTOZOOLOGY
JUNIOR MEDICAL LABORATORY SCIENTIFIC OFFICERS

re required to assist in research andidates should hold 'O' level passes in English, Maths, Chemistry and Biology, but preference will be given to those in possession of 'A' level Chemistry and Biology, or O N.C in Biological Sciences Salary, depending on age and qualifications, in the range 2,169 to £3,015 plus £354 London Weighting Applications, consisting of full education and career details and naming two referees, should be sent to Secretary (A1) at the School
9178(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
The Academic Department of Physiology requires a

TECHNICIAN
and a
JUNIOR TECHNICIAN

to work in the Clinical Science Building at the Royal Free Hospital in Hampstead.

Salary on Whitley Council Scales according to age and qualifications.

Application forms are available from the School Secretary, R F.H.S M, 8 Hunter Street, London WC1N 1BP, or telephone 01-837 5385, ext 10. Closing date: February 2, 1979
9243(A)

POSTDOCTORAL POSITION

available immediately for immunological studies of the mechanism of immune regulation by antibodies Research experience in transplantation immunology is preferred Appointment will be for one or two years

Applications with curriculum vitae and names of two referees should be sent to

Dr R A. P. Koene
University of Nijmegen
University Hospital St Radbound
Department of Nephrology
Nijmegen, The Netherlands
9239(A)

MEDICAL COLLEGE OF WISCONSIN
POSTDOCTORAL POSITION

Conformational attributes of proteins and synthetic model systems studied by kinetic, spectroscopic and other methods. Requires background in biophysical chemistry and/or peptide synthetic chemistry Available immediately

Salary $11,000 to $13,000

Send curriculum vitae and 3 letters of recommendation to Dr Henry E Auer, Department of Biochemistry, Medical College of Wisconsin, P O Box 26509, Milwaukee, WI 53226, U S A

Equal Opportunity/Affirmative Action Employer 9235(A)

UNIVERSITY OF MISSISSIPPI MEDICAL CENTER
POSTDOCTORAL POSITION

Research on the molecular biology of insect development, hormonal control of mRNA synthesis and in vitro transcription Experience in nucleic acid hybridisations desired

Send resume and names of three references to Dr S Sridhara, Department of Biochemistry, University of Mississippi Medical Center, 2500 North State Street, Jackson, Ms 39216

The University of Mississippi Medical Center offers equal opportunity in education and employment. Male/Female 9237(A)

MEDICAL RESEARCH COUNCIL
DUNN NUTRITION UNIT

Postdoctoral research worker required to undertake studies on energy balance in man Studies will be conducted in a new metabolic suite incorporating three whole body calorimeters at the Unit's Clinical Nutrition Centre, Addenbrooke's Hospital, Cambridge Experience in human physiology desirable, with or without a medical qualification The appointment is for three to five years, with salary according to qualifications

Further details and applications by February 15, 1979, to Dr W P T James, Dunn Clinical Nutrition Centre, Addenbrooke's Hospital, Trumpington Street, Cambridge CB2 1QE. 9186(A)

CSIRO AUSTRALIA
Research Scientist
Division of Land Use Research
Canberra, Act

CSIRO has a broad charter for research into primary and secondary industry areas The Organisation has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia

Field: Soil Science (Soil Hydro-Geography)

General: The Division is engaged in research on land and water resources and their evaluation for various uses. Its current research is concerned, *inter alia,* with development of methods of resource surveys as well as methods of predicting, from resource data, biological productivity and water flow and quality. The Division is therefore particularly interested in interpretation of soil data, which can be acquired through surveys and associated laboratory studies and presented in form of maps, in terms of hydrological properties needed to determine water balance aspects of the hydrological cycle and to estimate water availability to plants.

Duties The appointee will participate in collaborative research concerned with resource surveys and use of survey information for evaluating land capability for agricultural, pastoral and forestry uses and for engineering structures He or she will undertake the development of methods of determining, from survey and laboratory data, hydrological soil characteristics which will allow better quantitative prediction of hydrological cycle parameters, particularly through-drainage, run-off and soil water available to plants

Qualifications: Ph D or equivalent qualifications together with interest and experience in research on both soil physical and soil survey problems

Salary: Research Scientist or Senior Research Scientist $A14,829 to $A21,543 p a Appointment at higher level may be considered for suitably qualified candidates

Tenure Indefinite with superannuation.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 618/453 should reach—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 16th February 1979

Applications in U S A and Canada should be sent to—The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N W, Washington, D.C, 20036, U.S A. 9207(A)

LA TROBE UNIVERSITY
Melbourne, Australia
School of Biological Sciences
DEPARTMENT OF BIOCHEMISTRY
LECTURER

Applications are invited for this post from suitably qualified biochemists to teach in undergraduate courses, and to carry out research in co-operation with existing members of staff. Applicants should have a Ph.D in Biochemistry and relevant teaching experience The appointment will be for a fixed term of up to three years.

SALARY $A15,786 to $A20,737.

Further information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF Applications marked confidential and quoting reference number 150/14/19 close *February 9, 1979.* 9153(A)

UNIVERSITY OF MIAMI
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for the position of Research Associate in the Department of Biochemistry, University of Miami School of Medicine A biochemist with experience in immunological techniques and cell biology would be preferred Duties will include studies on procedures for enzyme modification that result in products with increased therapeutic usefulness [see, for example, *Trends in Biochemical Sciences* (1978) 3, 79–83]

Salary according to experience Only highly motivated individuals need apply.

Further information from and applications to Dr J. J Marshall, Department of Biochemistry D4-9, University of Miami School of Medicine, P.O. Box 016960, Miami, Florida *33101.*

Applications should include the names of three referees. 9236(A)

Royal Botanic Gardens, Kew

Botanist

...undertake taxonomic research on palms

The scientific work of the Royal Botanic Gardens is concerned with the classification, growth, structure and uses of plants, utilising the collections of preserved material in the Herbarium and Museums, and the unique collection of living plants in the gardens.

The Herbarium, contains about 5 million specimens, and is supplemented by a comprehensive library specializing in taxonomic literature. Included in this Herbarium is a rich collection of the important, yet often neglected, tropical family Palmae. In recent years, the palm specimens, including large sheets and carpological material, have been re-housed and the botanist appointed will curate this specialised collection and undertake taxonomic research on the Palmae.

Candidates, normally aged under 32, should have a good honours degree, or equivalent in Botany. At least 4 years relevant postgraduate experience is essential; experience of palms, particularly if gained in the field, is highly desirable.

Appointment as Senior Scientific Officer (£5425-£7175) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme. *Please quote ref: SB/17/AH.*

■ *For application details please see below.*

Home Office, Birmingham

Scientists

...forensic Studies of Documents

Scientists are required in the Document Section of the Forensic Science Laboratory, which carries out investigations for the Police relating to questioned documents and handwriting.

The successful candidates will be concerned with photographing documents by infra-red, ultra-violet and luminescence methods and the examination of paper, inks, handwriting and typescript for evidence. Initially, whilst training, they will assist senior colleagues, but will ultimately prepare reports and attend court hearings as expert witnesses.

Candidates, normally aged under 27, should have a good honours degree or equivalent in a scientific subject. Appointment as Scientific Officer (£2840-£4410) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme. *Final year students will not be considered. Please quote ref: SB/21/LA.*

For further details and an application form (to be returned by 9 February 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote appropriate ref.*

ALL THESE POSTS ARE OPEN TO MEN AND WOMEN.

9204(A)

Science Group
CIVIL SERVICE

RESEARCH ASSISTANT

A graduate chemist or biochemist with an interest in biochemical engineering is required for a pilot study of animal feed protein production from activated sludge. The appointment will be for one year, and the person appointed will be responsible for running the pilot plant and carrying out various analytical procedures.

The salary will be in the range of £2,800 to £3,400 depending on qualifications and experience.

Applications, including the name and address of one referee, should be sent to Dr R. A. Grant, 41A Springfield Road, Parkstone, Poole, Dorset.

9188(A)

MEDICAL RESEARCH COUNCIL

CELL BIOPHYSICS UNIT

There is likely to be, in the immediate future, a vacancy for a postgraduate research student to undertake work in one of the Unit's areas of activity (especially neurofilaments, muscle and membrane biochemistry, muscle structure and function). Remuneration and conditions as for normal Research Council studentships.

Apply in writing, together with the names and addresses of two referees, as soon as possible to: Professor M. H. F. Wilkins, CBE, FRS, Cell Biophysics Unit, 26-29 Drury Lane, London WC2B 5RL.

9187(A)

THE UNIVERSITY OF THE WEST INDIES—JAMAICA

Applications are invited for the following posts:

RESEARCH FELLOW (Physics)

Science Education Centre

Applicants should at least hold a first degree in Physics and have professional training for teaching. Preference will be given to those with experience of teaching in the West Indies. In addition it will be an advantage to have experience in testing, evaluation and assessment.

LECTURER/ ASSISTANT LECTURER in Inorganic Chemistry

The appointee will be expected to play a major role in the teaching and organisation of the Chemistry courses and to contribute to the research programme of the Department.

LECTURER IN ZOOLOGY (Marine/Fisheries Biology)

The appointee will be expected to assist in the development of programmes of research and teaching in marine biology and fisheries biology. The appointee must be able to take up the post not later than September 1, 1979. Candidates who responded to an earlier advertisement (March/April 1978) need not re-apply.

LECTURER/ ASSISTANT LECTURER in Botany

Preference wil be given to applicants who possess a first degree with majors in Botany and hold postgraduate qualifications in Plant Pathology. The appointee will be required to take up duties as soon as possible.

Salary scales: Research Fellow/ Lecturer J$8,913 to J$13,917 p.a.; Assistant Lecturer J$7,236 to J$7,926 p.a. (£1 sterling=J$3.46). F.S.S.U.; Study and Travel Grant. Unfurnished accommodation will be let by the University at a rental of 10% of salary or a housing allowance of 20% of salary will be paid. Family passages.

Detailed applications (2 copies) with curriculum vitae and naming three referees should be sent as soon as possible direct to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica.

Applicants resident in the U.K. should also send one copy to 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

9233(A)

JAMES COOK UNIVERSIT
OF NORTH QUEENSLANI
CHAIR IN GEOLOGY

The Chair in Geology will becor
vacant on August 1, 1979, on t
retirement of the foundation head
Department, Professor W. C. Lacy.

The appointee will be Head of t
Department of Geology which is acti
in teaching and research in aspects
Applied Geology 'Hard-Rock' Geolo
Palaeontology and Sedimentolo
Structural Geology, Marine Se
mentology and Geochemistry. Appli
tions are invited from persons with
interest in any of these fields.
addition to the usual undergradu
and postgraduate activities, the Depa
ment, under Professor Lacy, h
developed a special master program
Exploration and Mining Geology whi
involves considerable interaction wi
the Mining Industry. The Univers
would wish to see this activity ma
tained and developed.

Salary for Professor is curren
$A33,061 per annum. Conditions
appointment include F.S.S.U. ty
superannuation, invalid pensi
scheme, housing assistance and allo
ance for travelling and remo
expenses on appointment.

Additional information and applic
tion forms may be obtained from t
Registrar, Post Office, James Co
University, Qld 4811, Australia,
from the Association of Commo
wealth Universities (Appts), 36 Gord
Square, London WC1H 0PF.

Applications close on **February**
1979.

9232(A)

UNIVERSITY OF EXETER
DEPARTMENT OF CHEMISTR
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a Po
doctoral Research Fellow to work w
Dr P. M. Hardy on the synthesis
analogues of the decapeptide loc
adipokinetic hormone as part of
investigation into its structure/activ
relationships. Applicants for t
Science Research Council sponsor
post should have experience in sy
thetic organic chemistry. The appoi
ment will be for one year commenci
as soon as possible after March
1979.

Salary £3,883 or £4,133 per ann
depending on age and qualificatio

Applications, with a curricul
vitae and the names and addresses
two referees, should be forwarded
soon as possible to Dr P. M. Har
Department of Chemistry, Univers
of Exeter, Stocker Road, Exeter E
4QD from whom further informati
may be obtained.

9173(A

ANALYST TECHNICIAN (Grade
required for Department of Geolo
University of Reading. Preferen
given to applicants experienced in
chemical methods for silicate analy
but those with substantial experien
in general inorganic analysis by ator
absorption and Auto-Analyser te
niques also considered. Salary acco
ing to qualifications and experience
range £3,654 to £4,365 p.a. (un
review). Further details under R
T. 79A from Assistant Bursar (Pers
nel), University of Reading, Whi
knights, Reading RG6 2AH, to wh
applications should be sent to arr
not later than January 26, 1979.

9244(A

ANALYST TECHNICIAN (Grade
for Department of Geology, Wh
knights. Preference given to applica
experienced in wet chemical meth
for silicate analysis, but those w
substantial experience in gen
inorganic analysis by atomic abs
tion and Auto-Analyser techniq
also considered. Salary according
qualifications and experience in ra
£3,654 to £4,365 p.a. (under revie
Further details available, quoting
T.N. 79. Applications should be
to arrive not later than January
1979.

9245(A

DIRECTOR OF DEVELOPMENT U.S.A.

Important European pharmaceutical Company is looking for a Director of Preclinical and clinical R & D for its new American operation.

Should be Ph. D. or equivalent, experienced with regulatory affairs and phases I, II and III trials.

Unique opportunity for creating new team of clinical R & D throughout the U.S.A.

Please apply in confidence in your own handwriting and send your CV with identity photo to SNPM - 100 avenue Charles De Gaulle - 92522 Neuilly Cédex (France), under reference number 1528.

9241(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL

EPTIDE SECTION (DEPARTMENT OF CHEMICAL PATHOLOGY)

TECHNICIAN/SCIENTIFIC OFFICER

quired in the Peptide Laboratory to ke part in the synthesis of peptide rmone analogues Applicants should ssess H.N.C. or a degree in Chem- try and have some experience in ganic synthesis The post is tenable r 18 months with a possibility of rther extension.

Salary within the range £3,646 to ,199 placing dependent on qualifica- ons and experience.

Application forms and further par- ulars may be obtained from the rsonnel Office, Royal Postgraduate edical School, 150 Du Cane Road, ndon W12 0HS quoting reference umber 4/203/N 9202(A)

IOMEDICAL ELECTRONICS TECHNICIAN

A TECHNICIAN (male/female) is quired as part of a research team at e Hearing and Balance Unit Re- onsibilities will include design and nstruction of electrical equipment perience with sound reproduction/ gital computation/servocontrol sys- ms — an advantage The Unit nctions as a research and service partment as part of the hospital and e position will involve frequent con- t with patients

Recognised qualifications will be quired for establishment

Salary according to age and perience

Applicants should provide a written rriculum vitae and the names and dresses of three referees to: The rector, Medical Research Council, aring and Balance Unit, National spital, Queen Square, London CIN 3BG. 9198(A)

GUY'S HOSPITAL MEDICAL SCHOOL

(University of London)

POSTDOCTORAL RESEARCH ASSISTANT

required to join a research group under Dr L. Mallucci in the Department of Microbiology investigating the role of cell surface membrane components in cell growth regulation and cell differentiation Position available to December 1979 with the possibility of further renewal of grant.

Salary in range £3,910 to £5,506 plus £450 London Allowance and superannuation.

For further details telephone Dr Mallucci.

Applications to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT.

9055(A)

UNIVERSITY COLLEGE OF NORTH WALES

BIOCHEMISTRY

Applications are invited for the post of

PROFESSOR

of Biochemistry and Head of Department

The appointment will be from OCTOBER 1, 1979 (or a date to be arranged) and the salary will be in the professorial range

Further particulars can be obtained from the Secretary and Registrar, University College of North Wales, Bangor, Gwynedd LL57 2DG

Ten copies of the application (one from overseas candidates) together with the name of three referees should be sent to reach the Secretary and Registrar by Friday, February 16, 1979

9048(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellows

Division of Land Resources Management Alice Springs, NT and Deniliquin, NSW

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: SOCIAL SCIENCE

General: The Division has problem oriented research programs concerned with the environmental, societal, economic and technological implications of land use in range, agricultural, forested and near urban areas, and with the development of methods for processing and communicating data to assist land use decision making.

As a further expansion of our activities in these areas, we wish to appoint social scientists to join multi-disciplinary, but predominantly biological, research teams at Alice Springs and Deniliquin. These teams are concerned with research into the management of and and semi-and rangelands of Australia

We are seeking two quantitative scientists with research experience (Ph.D or equivalent) in at least one of the following fields:

Social Psychology
Environmental Economics
Quantitative Sociology
Human Geography

who will interact with research in progress at Deniliquin and Alice Springs The appointees will also interact with other social scientists located at Divisional headquarters in Perth, W.A.

Duties

Alice Springs—Ref No. 284/86

In association with technological scientists, the appointee will research social, economic and land tenure problems associated with the management of and lands

Deniliquin—Ref. No. 284/89

The appointee will research the way in which the pastoralists' perceptions of the land resources they manage is influenced by biological, economic, political and social variables. This research will relate to existing socio-economic research which is identifying the effect of land tenure and administration on pastoral management and will assist in developing processes which will improve and land management.

Salary: Research Scientist or Senior Research Scientist $A14,829 to $A21,543 p a An outstanding applicant may be offered appointment at a higher level

Tenure: A fixed term of two to five years (negotiable)

Applications IN DUPLICATE, stating FULL personal and professional details, the names and addresses of at least two professional referees, and QUOTING THE APPROPRIATE REFERENCE NUMBER(S) should reach. The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, London WC2R 3EH by 16th February 1979.

Applications in U S A and Canada should be sent to: The Counsellor (Scientific) Embassy of Australia, 1601 Massachusetts Avenue N W, Washington, D C 20036 9211(A)

CHELSEA COLLEGE

University of London

BIOPHYSICS

Grade 4 Technician required to assist in research and teaching activities within the Biophysics section of the Basic Medical Sciences Group Experience of Biophysical and/or Biochemical techniques would be an advantage Salary scale (under review) £3,420 to £3,867 per annum inclusive.

Enquiries and application forms from the Secretary, Biophysics Laboratory, Chelsea College, 17A Onslow Gardens, London SW7 3AL (01-589 4438)

9246(A)

UNIVERSITY OF QUEENSLAND

Australia

LECTURER IN PHYSIOLOGY

Preference will be given to applicants with research interests in endocrinology, reproductive physiology, fetal or developmental physiology *March 31, 1979*

Salary $A15,179 to $A19,940 per annum Other Benefits Superannuation, housing assistance, travelling and removal expenses.

Additional information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 9183(A) appointment of

Director

(male or female)

Brain Metabolism Unit

The Council invites applications for the post of full-time Director at the MRC Brain Metabolism Unit in Edinburgh The successful applicant will be a person of high scientific standing (with or without a medical qualification), with an established reputation as an original scientific investigator The Unit is at present engaged in basic neurobiological, and clinical research relevant to psychiatry and neurology The Council wishes the psychiatric interests of the Unit to be maintained The title and programme of the Unit will be decided by the Council in the light of the research interests of the new Director

The Unit is mainly housed in research accommodation of approximately 4,000 sq ft (nett) in the Department of Pharmacology of Edinburgh University and within the Royal Edinburgh Hospital An active research team is already in post· the size of the Unit will be a matter for discussion between the Director and the Council The Director will be responsible for maintaining the strong links already existing with the University of Edinburgh

The salary for a medically qualified Director, for whom an honorary clinical contract at consultant level will be sought, will be on the MRC clinical scale, equivalent to NHS consultant grade; if an honorary contract is not appropriate, the salary will be within the range for the Council's Special Appointments Grade, which is equivalent to the University professional range

MRC

Further information may be obtained from Miss R Withnall, 20 Park Crescent, London W1N 4AL Applications should be submitted to the Secretary of the Council at the same address, not later than 19 February, 1979

Medical Research Council

9146(A)

UNIVERSITY OF
STRATHCLYDE

Applications are invited
for the post of

LECTURER

in the

DEPARTMENT OF
PHARMACEUTICAL
CHEMISTRY

to teach physical and biophy
aspects of the subject to ho
and postgraduate students
pharmaceutical science Appli
should have experience of res
in the application of phy
chemistry to the study of
action.

Salary scale £3,883 to £
with placing according to qual
tions and experience Superan
tion benefit

Application forms and fu
particulars (quoting 4/79)
enclosing a self-addressed env
(9" x 4"), may be obtained
the Academic Appointments Of
University of Strathclyde, I
College Building, 204 G
Street, Glasgow G1 1XW,
whom applications should
lodged by February 5, 1979

9234(

WYE COLLEGE
(University of Londor

LECTURER IN LIPI
BIOCHEMISTRY

Applications are invited for tl
of Lecturer in Lipid Bioche
The post is a permanent one an
expected that the successful ca
would have considerable experie
research and teaching in this fi
is hoped that the Lecturer w
able to take up the post on A
1979 or as soon as possible the
Applications to be forwarded
Secretary, Wye College, Wye, A
Kent TN25 5AH (from whom
particulars of the post may
tained) by March 1, 1979 at the

916

Agrochemical
Field Evaluation

We are looking for a graduate with a good honours degree in Agriculture or related subjects to join a young and enthusiastic team conducting early stage field evaluation trials with new agrochemicals. Applicants should have post graduate experience in field experiments together with practical experience in arable husbandry. The person appointed will be based at Jealotts Hill Research Station, Bracknell, Berkshire but should be willing to travel extensively planning and organising the work of field teams based in the main areas of the U.K.

We offer good salaries and fringe benefits including membership of the ICI Pension Fund and Profit Sharing Schemes. A Staff Restaurant and Recreation Club are on site.

If you are interested in this vacancy please write for an application form to: Mr S R Stephenson, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berks.

Ref No 79/FE 9213(A)

ICI Plant Protection Division

EALING, HAMMERSMITH AND HOUNSLOW AREA HEALTH AUTHORITY (T)

CHARING CROSS HOSPITAL (FULHAN

MEDICAL PHYSICS DEPARTMENT

BASIC GRADE PHYSICIST

Applications are invited for the above vacancy in this teaching hospital. Applicants should have a first or second class Honours degree in Physics. The duties will be mainly concerned with the measurement and application of radiation
in the medical field. Applicants should also have a basic knowledge of physics, outside the field of ionising radiations. Experience in the use of computers would be an advantage.

Previous experience in hospital physics is essential.

Salary in accordance with Whitley Council regulations, within the range £4,152 to £5,253 including London Weightin

Application forms and further information available from District Personnel Department, Charing Cross Hospital, Fulham Palace Road, London W6 9HH. Tel: 01-748 2040, Ext. 2997.

Closing date for receipt of applications February 8, 1979.

9247(A

PLANT MOLECULAR BIOLOGIST

BROOKHAVEN NATIONAL LABORATORY

Position now available for plant scientist to conduct research in molecular biology Candidates must have a background in the plant sciences and demonstrated experience in the area of molecular genetics. Research initiated by the investigator may be at a fundamental level but must be directed toward the U S. Department of Energy's long range goals of increasing biomass productivity. This would include basic research on cellular or genetic mechanisms that control plant growth, productivity, and response to the environment

Applicants should submit a curriculum vitae (including list of publications), brief research proposal; and the names of three references to:

H. Z Kondratuk
Biology Department
Brookhaven National Laboratory
Associated Universities, Inc.
Upton, L.I., N.Y. 11973

An Equal Opportunity Employer M/F

9168(A)

Experimental Officer

Biochemistry Department

A Biochemist is required to work within the Natural Products Section of our Biochemistry Department The job will involve the development of biochemical screens to evaluate potential anti-inflammatory agents from natural sources and the subsequent follow up of active leads. The successful candidate will be expected to have a degree or H.N C. in biochemistry and experience of the pharmaceutical industry would be an advantage but not essential.

Our modern research laboratories are situated in pleasant surroundings, but within easy reach of main road and rail routes Conditions of service, salary and assistance to married staff in moving house are designed to attract and retain staff of high calibre

Please write giving details of qualifications and experience to:
Mrs D Bass,
Assistant Personnel Officer,
Imperial Chemical Industries Limited, Pharmaceuticals Division, Mereside Research Laboratories,
Alderley Park,
Nr. Macclesfield,
Cheshire.

ICI

9216(A)

British Museum Research Laboratory, London

GEOLOGIST

The British Museum comprises the national collections of Antiquities and Ethnography and of Prints and Drawings Its purpose is to advance learning by the provision of materials and facilities for research, and by encouraging the study of history, archaeology and art The Research Laboratory provides services and advice to other Departments of the British Museum in connection with the scientific examination and dating of objects in the departmental collections The Laboratory is equipped with excellent research facilities

The geologist appointed will undertake petrological examinations of ceramics and stone artefacts with a view to establishing the origin of the raw materials used, and the methods of ceramic manufacture This will involve participation in research projects using petrological examination, neutron activation analysis and scanning electron microscopy.

Candidates, normally aged under 30, should have a good honours degree or equivalent in geology and at least 2 years' relevant postgraduate experience which must include a significant element of petrology

Appointment as Higher Scientific Officer (£4,560 to £5,910) with starting salary according to qualifications and experience Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 9th February, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB or telephone Basingstoke (0256) 68551 (answering service operates outside office hours) Please quote ref.: SB/7/DJA.

9147(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellow

Division of Land Resources Management Alice Springs, NT

CSIRO has a broad charter for research into primary and secondary industry areas The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: RANGELAND ECOLOGY

General: The Division with headquarters in Perth, W A., is involved in research on the broad aspects of management of range, agricultural and forested lands in Australia. The Rangelands Research Program is based at Alice Springs, NT and Deniliquin, NSW and is responsible for research on the arid and semi-arid lands used for grazing, national parks, mining, and by Aborigines

Duties: The appointee will work in a multi-disciplinary team with five other professional scientists and will be expected to develop a research program on the ecology of key rangeland plant species.

Qualifications: A Ph.D or equivalent research experience in any field of quantitative plant ecology.

Salary: Research Scientist or Senior Research Scientist: $A14,829 to $A21,543 p a. An outstanding applicant may be offered appointment at a higher level.

Tenure: A fixed term of two to five years (negotiable)

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 284/87 should reach:

The Personnel Officer, Australian Scientific Liaison Office,
Canberra House, 10-16 Maltravers Street, London WC2R 3EH,
by 16th FEBRUARY 1979

Applications in U S.A. and Canada should be sent to:
The Counsellor Scientific, Embassy of Australia,
1601 Massachusetts Avenue, N.W , Washington, D C.,
20036, U.S.A

9209(A)

Virologist and Research Officer

NIBSC is a multidisciplinary Institute responsible for the testing and control of biological substances used in medicine and is also a WHO International laboratory for biological standards The work of the Institute is well supported by research.

We wish to appoint a Virologist (scientist) and a Research Officer to join the Institute's Division of Viral Products.

Virologist

(Ref 0178)

The Scientist appointed will work in the field of hepatitis and/or rabies. The successful candidate will have at least 3 years' postdoctoral experience in virology or a closely related field Preference will be given to those with research experience in either the Immunochemistry of viruses or viral biochemistry.

Salary will be on grades analogous to those for university lecturers and the tenure of the appointment will depend on the experience and qualifications of the successful candidate.

Research Officer

(Ref 0180)

The Research Officer will work in the field of the standardisation and control of rabies vaccines in close association with a scientist. He or she should have had at least two years' relevant postgraduate experience in virology. Preference will be given to candidates who have previous experience of work with rabies or other highly pathogenic viruses Candidates should possess a degree or equivalent in the biological sciences

Salary, depending on age and experience, on the scale £3,615 to £5,256, including London Weighting.

Both posts are superannuable.

Write or telephone for an application form and further details, quoting the post reference number to the Personnel Officer, National Institute for Biological Standard and Control, Holly Hill, Hampstead, London NW3 6RB. Telephone 01-435 2232, Ext. 211.

NIBSC

National Institute for Biological Standards and Control

9214(A)

UNIVERSITY OF EXETER

CHAIR OF PSYCHOLOGY

Applications are invited for a newly established Chair in the Department of Psychology from candidates with special interest in Developmental/Social Psychology. The post is tenable from October 1, 1979, or earlier. Salary will be within the agreed professorial range, currently £9,443 minimum, £11,055 average per annum (under review).

Further particulars should be obtained from the Academic Registrar and Secretary, University of Exeter, Northcote House, the Queen's Drive, Exeter EX4 4QJ. Closing date for receipt of application February 12, 1979. 9144(A)

UNIVERSITY OF LONDON
British Postgraduate Medical Federation

CARDIOTHORACIC INSTITUTE (Associated with the National Heart & Chest Hospitals)

CARDIAC METABOLISM RESEARCH LABORATORIES 50 Wimpole St., London W1

The Unit comprises a multi-disciplinary team of research worker and is dedicated to the study of the physiology, biochemistry, ultrastructure and pharmacology of the myocardium. The following positions are now vacant:

Non Medical Lecturer

with experience in the field of membrane structure and function Applicants should have a Ph.D. degree or equivalent and will be required to initiate research and co-ordinate with other sections of the team Previous experience in the myocardium is not essential.

Salary on an appropriate point of the University of London scale fo non-medical Lecturers, currently £3,883—£7,754 + £450 London Weighting. Further details available from Professor Peter Harri (telephone 01-486 3043)

Applications, including a curriculum vitae, list of publications an names and addresses of three referees, should be sent in duplicate t the Secretary, Cardiothoracic Institute, Fulham Road, London S.W.3 to arrive no later than 16th February, 1979

and

2 Research Assistants

One to undertake a project concerned with ischaemic changes in th myocardium and the other to undertake a project concerned with th identification of beta-receptors in myocardial tissue. Both project will be suitable for a Ph D. thesis and registration for this higher degre will be encouraged Each post is offered for a maximum of three year initially Starting salary £3,615 p.a. + £364 London Weighting Application forms available on request from the House Secretary Cardiothoracic Institute, 2 Beaumont Street, London W.1 (telephon 01-486 3043). Closing date 23rd February, 1979.

9185(A)

THE UNIVERSITY OF

TEXAS AT DALLAS

The biology programme has an opening at the Assistant Professor level. Applicants should be actively engaged in research in **Eukaryote Molecular Biology**, have at least two years postdoctoral experience, and show evidence of independent, significant and productive work

The university has an upper level undergraduate degree programme in Biology and a graduate (M.S, Ph D.) programme in Biology (with an emphasis on molecular biology) and an active research programme supported by a number of federal research grants The current faculty research lies mainly in the area of prokaryote molecular biology and the successful candidate would be expected to help extend this experience into the eukaryotic field of formal courses, supervision of student research, and developing an independent research programme Send resume to: Academic Search No. 206, The University of Texas at Dallas, P.O. Box 688, Richardson, TX 75080 Deadline for applications: March 31, 1979. Indication of sex and ethnicity for Affirmative Action statistical purposes is requested, but not required The University of Texas at Dallas is an equal opportunity employer 9248(A)

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IN THESE

PAGES

Phone:

01-831-6901

PEPTIDE RESEARCH

DELALANDE is an international pharmaceutical company actively engaged in research.

DELALANDE RESEARCH CENTRE has decided to create a department specialising in peptide research and is looking for a senior scientist to direct the research in this field.

Applicants should have considerable experience which may have been acquired in either industry or university and should be able to set a multidisciplinary group in peptide research. Knowledge of French is not essential.

DELALANDE RESEARCH CENTRE is situated in attractive surroundings close to Paris ans is within easy reach of main roads and rail routes.

Applications consisting of a curriculum vitæ and a list of publications should be sent in complete confidence to:

Dr. P. DOSTERT Research Director
Centre de Recherche DELALANDE
10, rue des Carrières
92500 RUEIL-MALMAISON
FRANCE

DELALANDE

9224(A)

HAVAS CONTACT

AVON AREA HEALTH AUTHORITY (TEACHING)

Bristol & Weston Health District (Teaching)

Applicants are invited for a

RESEARCH ASSISTANT POST

he post will be suitable for a
raduate or laboratory scientific offi-
er with the appropriate experience
nd qualifications. The project, funded
or three years only by the South
Vestern Regional Health Authority,
ill investigate Endorphin levels in
regnancy and will be carried out
intly by the Department of Anaes-
hesia, Bristol Maternity Hospital,
nd the University Department of
harmacology.

Starting salary will be on the Whit-
y Council scale for Medical Lab-
ratory Scientific Officers or Graduate
cientific Officers and will be in the
gion of £2,991 to £3,261 according
the candidates qualifications and
xperience.

For further information please con-
ict Dr Trevor Thomas, Consultant
naesthetist, Department of Anaes-
hesia, Bristol Maternity Hospital,
elephone 22041, extension 5775.

Application forms are available
om Miss M. Woodsford, District
ersonnel Department, 10 Marl-
orough Street, Bristol BS1 3NP, to
hom completed applications should
returned by February 1, 1979.

9174(A)

NIVERSITY OF WARWICK

RESEARCH TECHNICIANS

The Department of Biological
ciences has vacancies in the Chloro-
ast and Developmental Biology
esearch Groups. The work involves
e isolation and characterisation of
roteins and nucleic acids, using
andard biochemical techniques. The
osts are for contract periods of one
three years. Applications are invited
om graduates and others with
ppropriate qualifications in bio-
hemistry and/or microbiology.

Salary either on the Technician
rade 2B scale: £2,529 to £2,880 p.a.,
r Grade 3 scale, £2,688 to £3,060
a. (both under review).

Application should be made by
etter quoting Ref: 24/D/79, giving
ull details of age, qualifications,
xperience, etc., and addressed to the
ersonnel Office, University of
Varwick, Coventry CV4 7AL, by
anuary 25, 1979.

9231(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

GRADUATE RESEARCH ASSISTANT

required for a three-year Wellcome Trust project in the Department of Physiology to assist with laboratory research on reproductive neuro-endocrinology.

Salary scale £3,384 : £3,720 : £3,975 (under review) plus £450 per annum London allowance.

Application forms available from the Establishment Officer, St. George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE.

9200(A)

UNIVERSITY OF WESTERN AUSTRALIA

Perth

Applications are invited for appointment to the

CHAIR IN PHYSICS

This is one of two established Chairs in the Department of Physics, the other being in Theoretical Physics (Professor M. J. Buckingham). Applicants should have teaching and research interests in Experimental Physics. The present intention is that the appointee will be Head of the Department for an initial period of three years. The current salary for a Professor is $A33,061 per annum. Benefits include superannuation similar to F.S.S.U., fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave and housing loan scheme. Further information including conditions of appointment may be obtained from the Staffing Officer in the University, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications in duplicate stating full personal particulars, qualifications, experience and the names and addresses of three referees should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009, by March 17, 1979.

9181(A)

SCIENCE MUSEUM

DEPARTMENT OF PHYSICS

Assistant Keeper

To organise work on a new gallery devoted to Atomic Physics and Nuclear Power, which will involve the challenge of presenting and interpreting these subjects to the general public. Other work includes responsibility for one or more of the Departmental collections including Heat, Electricity and Magnetism, Mechanics and Optics. Additional work entails production of scholarly catalogues, augmenting the collections by suitable acquisitions, preserving and conserving the objects.

Candidates should normally have a degree with 1st or 2nd class honours in Science or the History of Science or equivalent qualification. A broad interest in Physics or the History of Physics essential. Reading knowledge of French or German advantageous.

SALARY: as AK I £5,855 to £8,820 or AK III £3,575 to £5,305. Starting salary and level of appointment according to age, qualifications and experience. Non-contributory pension scheme.

For further details and an application form (to be returned by 9th February, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote ref.: G(2) 382.

9143(A)

NEWCASTLE AREA HEALTH AUTHORITY (TEACHING)

ROYAL VICTORIA INFIRMARY

CYTOGENETICIST

SCIENTIFIC OFFICER, BASIC GRADE

Graduate required immediately for work in active cytogenetics service laboratory involved in all aspects of diagnostic cytogenetics including leukocyte culture, amniotic fluid cell culture, fetal cultures, and bone marrow analysis. Previous experience preferable but not essential.

For further information please contact Professor D. F. Roberts, Telephone 0632 : 25131, extension 658.

Salary on Whitley Council P.T.A. Scales:

Probationary 1st or 2nd Class Honours degrees:
£3,486 by 5 increments to £4,275 per annum.

Other degrees:
£2,991 by 9 increments to £4,275 per annum.

Post-Probationary Scale:
£3,798 by 9 increments to £4,899 per annum.

Applications giving full personal details, together with the names and addresses of two referees, should be sent to the Senior Administrative Assistant (Personnel), Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP. Closing date January 29, 1979. Quote Ref. No. 79/5.

9159(A)

Microbiologist £2,839-£6,898

Applications are invited for a pensionable post in the Department of Agriculture's Agricultural and Food Bacteriology Division, Newforge Lane, Belfast.

The successful candidate will be expected to undertake research and investigational work connected with the microbiology of foods and may also be required to undertake lecturing duties in the Faculty of Agriculture and Food Science, Queen's University, Belfast.

Appointment may be at Senior Scientific Officer, Higher Scientific Officer, Scientific Officer level.

SENIOR SCIENTIFIC OFFICER

Over 25 and under 32 years of age on 31st December, 1979 with a 1st or 2nd class Honours Degree in an appropriate Science subject, Agricultural Science, Food Science or Agriculture, and at least 4 years appropriate postgraduate experience.

HIGHER SCIENTIFIC OFFICER

Under 30 years of age on 31st December, 1979 with an Honours Degree as above and at least 2 years appropriate postgraduate experience in Microbiology.

SCIENTIFIC OFFICER

Under 27 years of age on 31st December, 1979 with an Honours Degree as above. Where the applicant has no relevant postgraduate experience in Microbiology, preference may be given to those whose degrees have Microbiology as the main subject.

Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

The Civil Service Commissioners may decide to interview only those applicants who appear from the information available (including level of academic attainment and relevant experience) to be best qualified.

SALARY SCALES

Senior Scientific Officer — £5,154-£6,898
Higher Scientific Officer — £4,101-£5,448
Scientific Officer — £2,839-£4,415

Grading and starting salary will be related to qualifications and experience.

Please write or telephone for an application form quoting job reference SB 34/79/N to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 Ext 257). Completed forms must be returned to arrive not later than 1st February, 1979. 9157(A)

NORTHERN IRELAND CIVIL SERVICE

THE UNIVERSITY OF MANITOBA

invites applications and nominations for the position of

DEAN OF THE FACULTY OF SCIENCE

The Faculty of Science at The University of Manitoba has a complement of 180 faculty and 90 support staff. Its twelve departments and units span the Biological, Mathematical and Physical Sciences; they offer a full range of undergraduate Science programmes for approximately 2,100 students enrolled in the Faculty, courses for a large number of students from other faculties and schools and M.Sc. and Ph.D. programmes for about 280 graduate students.

Candidates should have achieved distinction as scholars in a major field of Science, have a commitment to advancing higher education in Science and have strong leadership ability. Relevant administrative experience and knowledge of the Canadian educational system would be assets.

The appointment is expected to commence September 1, 1979, and will be for a renewable term of normally five to seven years.

Please address responses to:

Provost R. A. Johnson,
Chairman,
Selection Committee for Dean of the Faculty of Science,
Room 202, Administration Building,
The University of Manitoba,
Winnipeg, Manitoba,
Canada, R3T 2N2.

Applications, nominations and suggestions will be received until March 15, 1979. 9171(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellow

Division of Mineral Chemistry
Port Melbourne, Victoria

CSIRO has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: SURFACE CHEMISTRY OF COAL

General: The work will be centred at the laboratories of the Division of Mineral Chemistry in Melbourne (Port Melbourne) but will involve collaboration with the CSIRO Fuel Geoscience Unit in Sydney (North Ryde). A period will be spent in Sydney at the Fuel Geoscience Unit and coal washeries on the NSW coal-fields.

Duties: To study the surface properties of Australian black coals in relation to the behaviour of coal constituents during the upgrading of coal by froth flotation.

Qualifications: Ph.D in Chemistry or equivalent. Experience in surface chemistry or applied colloid science will be an advantage.

Salary: Within the range Research Scientist to Senior Research Scientist: $A14,829 to $A21,543 p.a.

Tenure: 12 months' appointment with the possibility of extension.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 430/390 should reach—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 16th February 1979.

Applications in U.S.A. and Canada should be sent to—The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 9210(A)

Nature Conservancy Council

FIELD UNIT LEADER

SCOTLAND (Senior Scientific Officer Level)

Salary Scale: **£5,154—£6,898**

Applications are invited for the post of Field Unit Leader for Scotland, based at the Nature Conservancy Council's Headquarters for Scotland, in Edinburgh.

Background

The function of the FIELD UNIT is to develop a co-ordinated programme of survey and monitoring in SCOTLAND in conjunction with Country HQ, regions and the Chief Scientist by carrying out appropriate survey and monitoring projects and to synthesise and interpret the results so as to facilitate maximum use of the information by all staff within the organisation.

The Post

The successful applicant should be a biologist with considerable experience of ecological survey work who will be required to lead the newly formed Field Survey Unit in Scotland (outlined above). The work will involve surveys of the flora and fauna of particular habitats, assessing the impact of developments and monitoring changes in the environment.

Age and Qualifications

Preference will be given to candidates with experience of survey methodology and numerical data processing and with a wide knowledge of British vegetation types.

Candidates should have a *first or second class honours degree* or equivalent in biology, botany, zoology, geology or geography and should normally be aged at least 25 and under 32 years of age and have at least 4 years relevant post-graduate experience.

This post will entail extensive travelling and possession of a current driving licence is therefore essential.

Application forms and further details are available from Recruitment Section, Nature Conservancy Council, Godwin House, George Street, Huntingdon, Cambs PE18 6BU (Tel. 0480 56191). Closing date for completed application forms is February 16, 1979. Interviews will be held in March 1979. 9203(A)

Northern Ireland Forensic Science Laboratory

Scientific Staff

Applications are invited for the following pensionable posts in the Northern Ireland Forensic Science Laboratory, Newtownbreda Road, Belfast.

The successful candidates will join a team of scientists engaged in the scientific investigations of crime throughout Northern Ireland. The work is interesting and varied and offers wide scope for developing interests in the varied aspects of forensic science.

METALLURGIST

(Higher Scientific Officer)
£4101 - £5448
(Ref SB 28/79/N)

Applicants must be under 30 years of age at 31 December 1979 and possess:
(a) a 1st or 2nd class honours degree in Metallurgy; or
(b) an equivalent qualification acceptable to the Civil Service Commissioners.

Candidates must have at least 2 year's postgraduate experience in research or industry.

DOCUMENT EXAMINER

(Scientific Officer)
£2839 - £4415
(Ref SB 29/79/N)

Applicants must be under 27 years of age at 31 December 1979 and possess:
(a) a 1st or 2nd class honours degree in a relevant scientific subject; or
(b) an equivalent qualification acceptable to the Civil Service Commissioners.

The officer appointed will be involved in the comparison of handwriting and typewriting and the examination of signatures and documents to detect forgery, alterations, etc. Previous experience in this field may be an advantage.

SCIENTIFIC OFFICER

£2839 - £4415
(Ref SB 30/79/N)

Applicants must be under 27 years of age at 31 December 1979 and possess:
(a) a degree in pure or analytical chemistry; or
(b) an equivalent qualification acceptable to the Civil Service Commissioners.

Exceptionally, applications may be considered from candidates over the age limits who have specialised experience.

Starting salary will be related to qualifications and experience.

There are promotion prospects to Higher Scientific Officer £4101-£5448, Senior Scientific Officer £5154-£6898 and Principal Scientific Officer £6609-£8461.

Please write or telephone for an application form quoting the appropriate reference number to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (Telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than 1 February, 1979. 9238(A)

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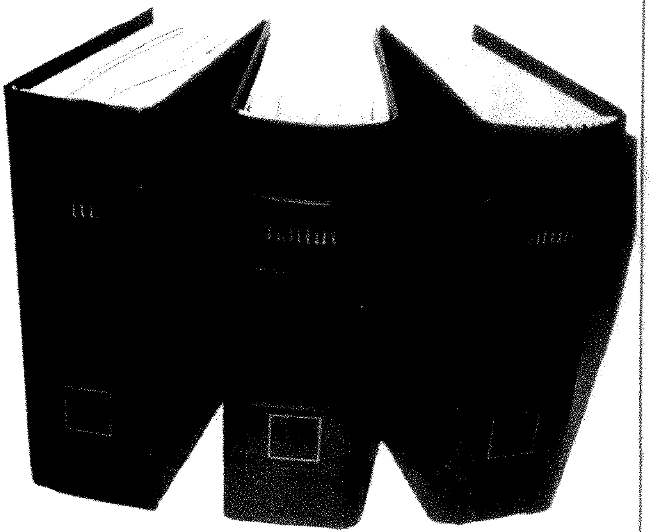

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CSIRO AUSTRALIA

Division of Applied Geomechanics Syndal, Victoria

CSIRO has a broad charter for research into primary and secondary industry areas The Organisation has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

1. Research Scientist

Ref. No. 920/247

Field: Slope stability applied to mining.

General: The Division is engaged in research on the engineering properties of soils and rocks and the application of this research in the fields of coal and metalliferous mining and urban, regional and marine geotechnology. The Division has its headquarters and main laboratories at Syndal near Melbourne Other staff are located in Adelaide, Sydney and mining areas in New South Wales and Queensland.

Duties: To supervise a research team engaged on studies related to surface mining. Initially the appointee will be required to conduct research aimed at defining the causes of slope failure in surface coal mines and predicting the mechanisms of failure at greater mine depths. Research will involve studies of the geology, material properties and stress in coal and adjacent strata together with the development of modelling techniques for surface coal mines.

Qualifications: A PhD degree or equivalent qualification in civil engineering, mining engineering or engineering geology, together with demonstrable research ability in one or more of the following fields: geology, hydrology, slope kinematics, modelling and behaviour monitoring

Location: The appointee will be located at Syndal, Victoria; however extensive field trips to the Bowen Basin, Queensland, and Hunter Valley, NSW, are envisaged.

Salary: Appointment will be made within the ranges of Senior Research Scientist or Principal Research Scientist · $A18,819-$A25,354 per annum

Tenure: Indefinite with superannuation

2. Postdoctoral Research Fellow

(2 positions)

POSITON 1—Ref. No. 920/245

Field: Engineering geology.

Duties: To initiate research into the structural aspects of roof instability in coal mines using geological and engineering techniques. Structural geology relevant to the support of laminated mine roof will be a substantial component of this study and initial field work will be conducted in the Sydney Basin.

Qualifications: A PhD degree or equivalent qualification in engineering geology or related discipline together with post graduate experience in this field. Experience in coal mining and/or familiarity with geomechanical mathematical modelling techniques as well as instrumentation techniques would be advantageous

Location: Depending upon circumstances the appointee to this position may be located either at Syndal Vic or at North Ryde, NSW.

POSITION 2—Ref. No. 920/246

Field: Mining engineering.

Duties: To conduct a review of underground mining methods potentially applicable to the Australian coal mining industry Particular attention is to be paid to typical Australian strata conditions such as geology, ground stress, seam and strata deformability and gas conditions Other duties will include participation in the development of physical and mathematical modelling techniques to be applied in the detailed evaluation of selected mining methods An involvement in the development of geomechanical monitoring techniques for use in underground coal mines environments is also anticipated

Qualifications· A PhD degree or equivalent qualification in mining engineering or related discipline with experience of operations in the coal mining industry

Location: Syndal, Victoria

Salary: Appointments will be made within the salary ranges of Research Scientist or Senior Research Scientist. $A14,829-$A21,543 p a Consideration will however be given to appointment at a higher level for outstanding applicants

Tenure: These positions are available initially for 12 months

Applications **in duplicate, stating full personal** and professional details, the names and addresses of at least two professional referees, and **quoting the appropriate reference number(s) should reach**· The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2R 3EH, by **18th March 1979.**

Applications in U S A. and Canada should be sent to The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue N W, WASHINGTON D C 20036 9229(A)

FELLOWSHIPS

UNIVERSITY OF WARWICK POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a postdoctoral research fellowship in the Departments of Chemistry and Molecular Sciences and Psychology, to work on an M R C sponsored project on specific anosmia and related areas of olfaction. The work will involve a systematic study of olfactory responses to urinous and musk odorants The project could suit either a chemist (biochemist) with an interest in phychophysics or a psychologist familiar with chemical methods.

The post is for two years, beginning as soon as possible, initial salary will be in the range £3,883 to £4,382 per annum on the Research range IA scale.

Applications, enclosing a curriculum vitae, details of research experience and the names of two referees should be sent to the Academic Registrar, University of Warwick, Coventry CV4 7AL as soon as possible Please quote Ref. No: 23/A/79. 9176(E)

QUEEN'S UNIVERSITY
Kingston, Ontario, Canada

Applications are invited for a postdoctoral fellowship to study the genetics and replication of insect baculoviruses The position is supported by M R C Canada for up to two years from receipt of a Ph D degree Stipend for the first year is $11,600 The position is available from April 15, 1979 The desirable qualifications are a background in virology and biochemistry of nucleic acids and proteins.

Please address inquiries to Dr Peter Faulkner, Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, K7L 3N6, Canada 9169(E)

The School of Pharmacy UNIVERSITY OF LONDON

TEACHING FELLOWSHIP IN PHARMACOLOGY

Applications are invited for this appointment at or about September 1, 1979, from suitably qualified persons

For the Teaching Fellowship, which is of limited term, postgraduate experience should relate to the research interests of the Department but additional experience in neuroanatomical, radio immunoassay and receptor binding assay techniques would be very welcome

Normal non-clinical university academic terms of appointment and salary scale Further information can be obtained from Professor D W Straughan

Applications to be submitted by February 9, 1979, in the form of letter accompanied by a C V and the names of two referees to:

The Personnel Officer,
The School of Pharmacy,
29/39 Brunswick Square,
London WC1N 1AX. 9201(E)

NEW ZEALAND UNIVERSITY OF CANTERBURY
Christchurch

Applications are invited for
University Postdoctoral Fellowshi
the Department of Civil Engineer
Applicants must possess a Ph D.
equivalent degree, preferably follo
by some research experience
fields within which the Fellow
may be held are. Construction
gineering, Fluid Mechanics, High
Engineering, Materials, Photogr
metry and Surveying, Public He
Engineering, Seismology, Soil M
anics, Structural Mechanics inclu
reinforced and prestressed conc
masonry, steel and timber struct
and Systems Engineering

Applications close on *March*
1979.

The emolument will be NZ$8
per annum. Fellowships are ten
for one year.

Further particulars and Condit
of Apopintment may be obtained f
the Association of Commonwe
Universities (Appts), 36 Gor
Square, London WC1H 0PF
9182(

AUSTRALIAN NATIONA UNIVERSITY

RESEARCH SCHOOL OF CHEMISTRY

PROFESSORIAL FELLOWSHIP IN ORGANOMETALLIC CHEMISTRY

Applications are invited for app
ment as Professorial Fellow in
area of Organometallic Chemistry

Founded in 1967, the Rese
School of Chemistry is an Integr
School without departments A
fessorial fellow can devote almost
whole effort to research, being
pected only to attend to m
administration in the School and
play some part in University aff
The School has no responsibility
undergraduate teaching It carries
research and teaches graduate stud
is well equipped by modern stand
and is well supported by technical
administrative staff The present D
of the School is Professor D P C
F A.A, F R.S

The School has a total acad
staff of seventy-one, of whom four
hold tenured appointments There
three professors (Professors A.
Birch, D P Craig and A M S
son) and two professorial fellows
J Ferguson and Mr R W Ricka
Professor B G Hyde of the Un
sity of Western Australia has rec
accepted a Chair of Chemistry in
area of Inorganic Chemistry

In the inorganic area there are t
tenured staff members leading i
pendent research groups (Prof
A. M Sargeson, Dr M A Ben
and Dr G B Robertson) They
supported by research fellows
Ph D students It is expected
Professor Hyde will take up
appointment early in 1979 and
an appointment will be made soo
the recently advertised fellows
senior fellowship in inorg
chemistry

The salary is $A29,532 per an
Current exchange rates $A1 e
58p $US1 14

Superannuation benefits are a
able The University meets reaso
travel and removal expenses for
appointee and his family Assi
with housing is provided for
appointee from outside Canberra.
The University reserves the righ
to make an appointment or to
an appointment by invitation at
time.

Further information about
position should be obtained from
Association of Commonwealth Un
sities (Appts), 36 Gordon Sq
London WC1H 0PF.

Applications close on *March*
1979. 9180

FELLOWSHIPS continued

THE UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF ANATOMY
POSTDOCTORAL RESEARCH FELLOWSHIP

A postdoctoral biochemist is re-uired to join a multi-disciplinary am studying aspects of growth and ifferentiation. The successful candi-ate will be responsible for a new I.R.C.-funded project involving the se of monoclonal antisera to study rotein polymorphism in muscle. echnical support will be available. he post is for three years beginning s soon as possible. Maximum starting alary will be £3,883 (plus superannua-on) on the Research Fellow 1A scale.

Applications (three copies), enclos-g a curriculum vitae, details of search experience and the names of ree referees, should be sent to the ssistant Registrar, Faculty of Medi-ine, University of Birmingham, irmingham B15 2TJ, from whom urther information may be obtained. losing date February 16, 1979. Please uote reference RF/ANT/478.

9221(E)

A RESEARCH FELLOWSHIP

tenable for a period of up to three years, is available for work on the regulation of eukaryotic genes by steroid hormones. Emphasis will be given on the isolation, cloning and characterisation of specific genes. Candidate should be experienced in the techniques of nucleic acid biochemistry. Applications (with curriculum vitae) should be sent to Dr J.-P. Jost, Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland.

9227(E)

UNIVERSITY OF KENT AT CANTERBURY
BIOLOGICAL LABORATORY
PROTOZOOLOGY GROUP
Applications are invited for a
POSTDOCTORAL RESEARCH FELLOWSHIP

work on the biochemistry of rimidine biosynthesis in *Trypano-na cruzi*. Preference will be given candidates with experience in zymology. Training in protozoo-gical techniques will be provided.

Salary range £3,883 to £4,882. The pointment is financed by the World ealth Organisation's Special Pro-amme for Research and Training on opical Diseases and will be for one ar initially, with good prospects of tension for a further period of two ars.

Application forms and particulars ay be obtained from the Senior ssistant Registrar, Faculty of Natural iences, Chemical Laboratory, The iversity, Canterbury, Kent, to whom mpleted applications should be sub-tted by February 7, 1979. Please ote Ref: A1/79. 9230(E)

STUDENTSHIPS

NIVERSITY OF LEICESTER
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a A.S.E. research studentship to work the control of aerobic energy con-vation in bacteria during growth in ntinuous culture. The project will supervised by Dr C. W. Jones niversity of Leicester) and Dr J. nton (Shell Research Ltd, Sitting-urne). The studentship is tenable m October 1979 and the successful ndidate will be expected to read for higher degree. Applicants should ssess, or expect to obtain, a good nours degree in Biochemistry or crobiology and should write to Dr W. Jones, Department of Bio-emistry, University of Leicester, icester LE1 7RH enclosing a cur-ulum vitae and the names of two erees. 9101(F)

COURSES

UNIVERSITY OF ABERDEEN
M.Sc. IMMUNOBIOLOGY COURSE 1979-80

Intending applicants for this 12 month course should apply for further details and application forms to Dr J. B. Solomon, Immunology Unit, Department of Bacteriology, University of Aberdeen, Aberdeen AB9 2ZD, as soon as possible. 9190(D)

ASSOCIATESHIPS

HERIOT-WATT UNIVERSITY
DEPARTMENT OF PHYSICS
POSTDOCTORAL RESEARCH ASSOCIATESHIP IN SOLID STATE THEORY

Applications are invited for an S.R.C. Postdoctoral Research Associateship, for studies of Anderson localisation and other topics in the theory of disordered solids, in association with Dr D. Weaire. The starting date should be no later than October 1, 1979.

Informal enquiries can be addressed to Dr Weaire.

Initial salary will be in the range £3,883 to £4,382 per annum (under review) of Range 1A.

Application forms are obtainable from the Staff Officer, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX. Ref. No. 22/78.

9196(O)

SCHOLARSHIPS

WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)
DEPARTMENT OF TRAUMATIC AND ORTHOPAEDIC SURGERY
Applications are invited for a
RESEARCH SCHOLARSHIP

tenable for up to three years from February 1, 1979, in the above Department at the Cardiff Royal Infirmary.

The project involves studies of the bone morphology and morphometry in some common fractures and in human osteoarthritis. The work, which will be done in the Orthopaedic Research Laboratories, will be closely integrated with the use of modern techniques such as microradiography and bone auto-fluorescence, as well as recently-developed special staining techniques. Studies of experimental material might also be involved.

Applicants should hold, or expect to obtain in 1979, a good Honours degree in basic science and be keen to work in the biomedical field and interested in the morphology of hard tissues. An ability to do some simple microscopical observations or some experience with the basic histological techniques would be an advantage. On the other hand, this Scholarship would give the applicant a good foundation for independent studies in the morphology of bone tissue. The appointee to be registered for a Higher Degree with the University of Wales, if suitably qualified.

Conditions are those pertaining to Tenovus Scholarships. Applications, which should take the form of a curriculum vitae with the names and addresses of two referees, should be submitted to Mr Z. Ralis, Senior Lecturer, Department of Traumatic and Orthopaedic Surgery, Cardiff Royal Infirmary, Newport Road, Cardiff, quoting Ref. No. T27/3/9. 9175(H)

SYMPOSIA

CALL FOR PAPERS
EUTROPHICATION IN ESTUARIES

An international symposium on

The Effects of Nutrient Enrichment in Estuaries

will be held on May 29, 30 and 31, 1979, in Williamsburg, Va. The conference is being organised by the Chesapeake Research Consortium and sponsored by the U.S. Environmental Protection Agency. One to two page abstracts for papers on relevant topics should be submitted by February 15, 1979, to:

Dr Bruce J. Neilson,
Virginia Institute of Marine Science,
Gloucester Point, VA 23062.
Phone: 804-642-2111.

9172(M)

LEUKAEMIA RESEARCH FUND
International Research Symposium on
PHILADELPHIA CHROMOSOME POSITIVE LEUKAEMIAS

will be held at the Royal College of Physicians, London, on **Tuesday, April 10, 1979.** Registration fee (includes lunch) £6. Apply for details and registration form to:

Leukaemia Research Fund,
43 Great Ormond Street,
London WC1N 3JJ
Tel: 01-405 0101

9155(M)

How Britain runs its science

This highly successful chart, which appeared in Nature 28 August 1975 (but is still much requested and much referred to), has now been completely revised and updated to include all major changes in the personnel and administration of British science.

Measuring approximately 2' x 3' and containing black-and-white illustrations of prominent figures, copies of this chart are now available at 75p (UK), US $1.50 (Rest of World).

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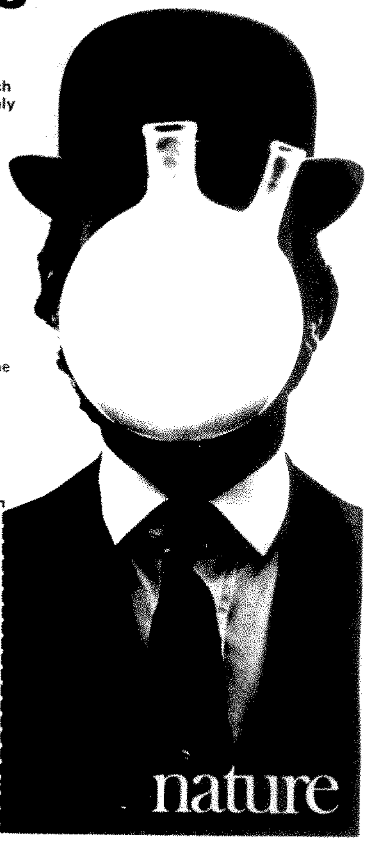
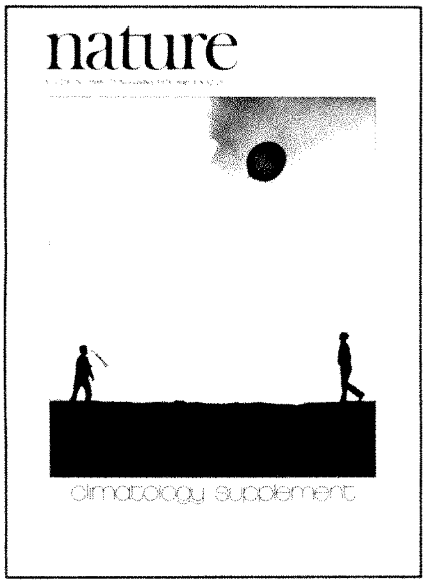


nature special supplement

Climatology (23 November 1978)



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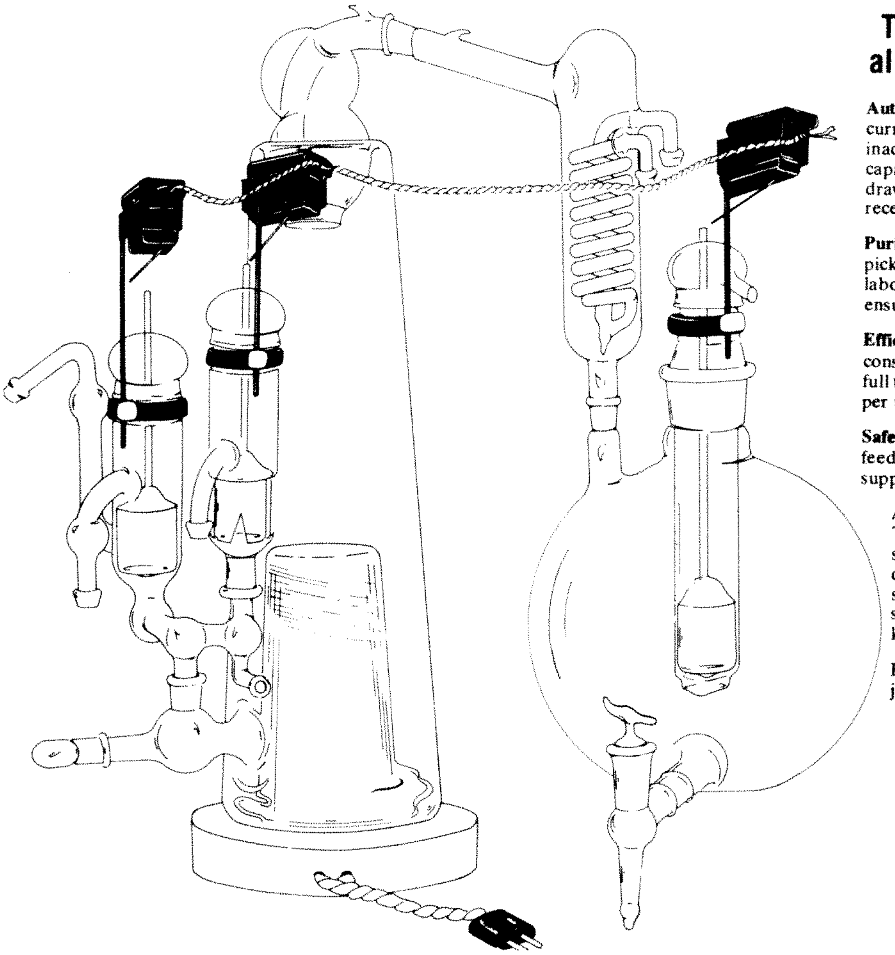

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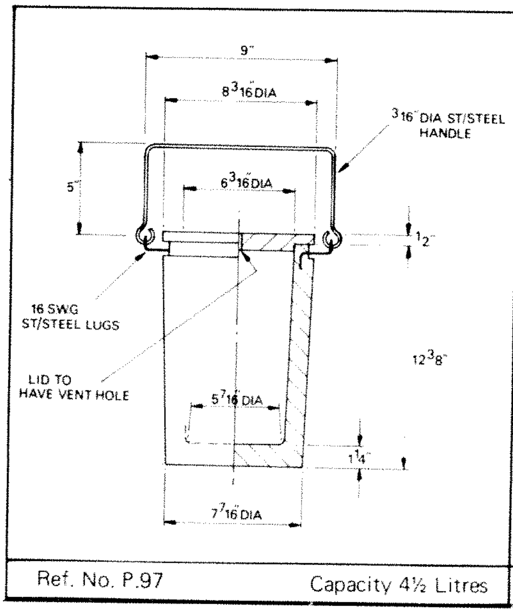

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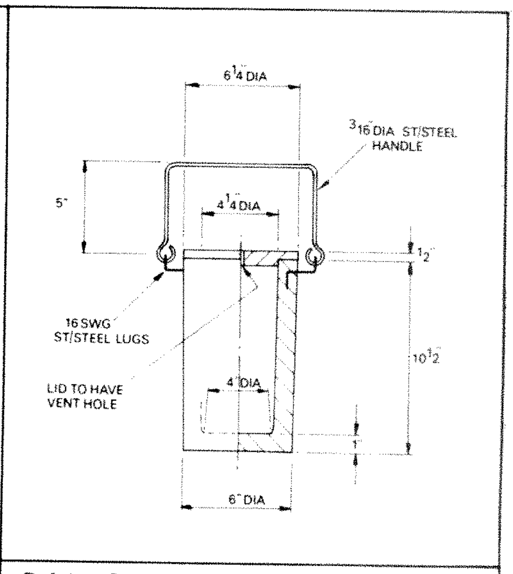

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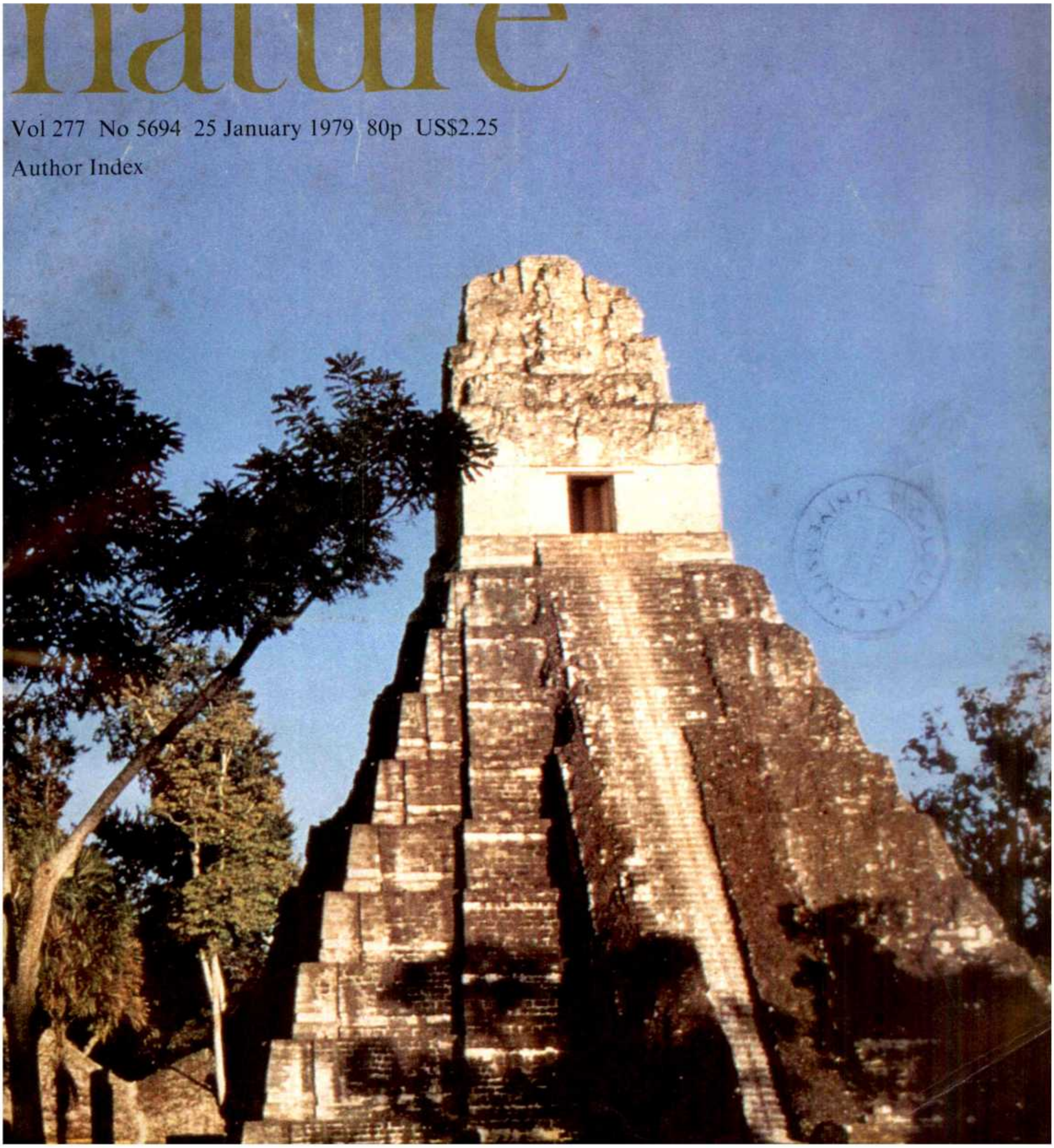

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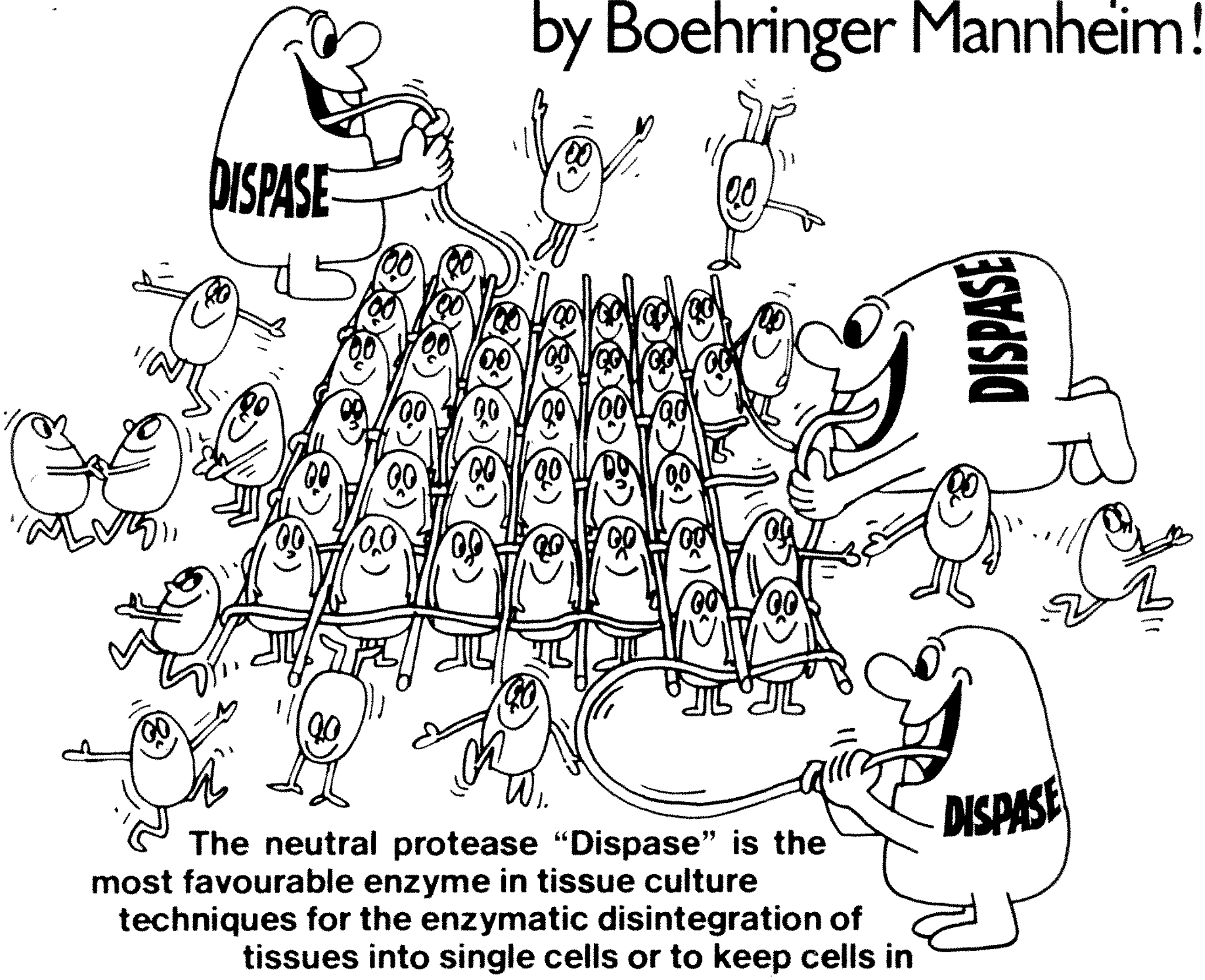

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Tikal, Peten, Guatemala. The North Acropolis and Temple I at sunset. On page 269 radiocarbon dating of the Late Classic Maya collapse is discussed. (Photo: D. M. Hales).

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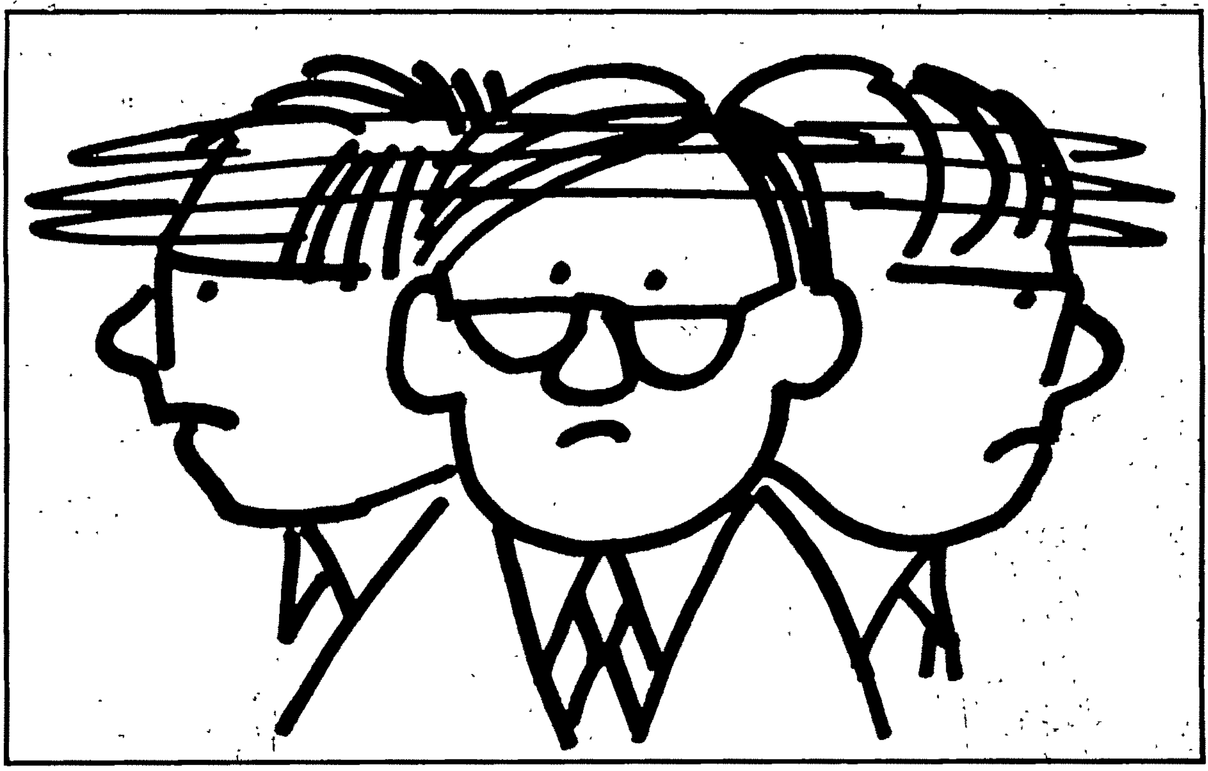

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25 January 1979

Converting intelligence into wisdom

DOES Britain use its intellectual resources wisely? There can be little doubt that the British education system has proved itself competent in the past at identifying and nourishing the high flier, from primary school through to university. Even in this time of egalitarianism there still seems a recognition of the need to sustain the outstanding student who may contribute something of exceptional value to society in the future. Twenty years of education can turn out people whose intellectual skills have been polished and sharpened to a high degree. But do we provide the right environment, as a nation, to capitalise for the next forty years on these skills; not just for the benefit of an organisation but also so that the individual may continue to feel a sense of fulfilment from being intellectually extended?

There is a trivial sense in which every educational system is immensely wasteful, and must be so to be effective. The vast majority of what is learnt in school or university is never specifically going to be used later in life but can only at best be described as intellectual compost—material which helps to explain 'the way things are' and which may illuminate future thinking in an undefinable way. But there is a far less trivial, and far more nation-specific way in which intellectual resources may be used or squandered. If the structure of a society is so heavily compartmentalised that the only way ahead is to become a specialist, and progressively to acquire greater expertise in a narrower discipline, then there is a small chance that intelligence will lead to wisdom. If there is an element of cross-movement open, at all levels, there is at least the possibility that every year will bring sounder judgement and more job-satisfaction.

Britain is indubitably a compartmentalised society: civil servants, academics, lawyers, doctors, politicians, industrialists and many others—they all pursue their own separate and highly specialised paths, defending themselves against outsiders and looking askance on those few who choose to step off a well-defined ladder.

The civil service is as good, or bad, an example as any. Competition for entry into the service is fierce; very intelligent people enter the service in their twenties. Whatever the label they carry—administrative, scientific and so on—they can be reasonably sure of a lifetime of employment, and indeed very few ever think of changing career. Certainly during that lifetime for many there will be varied work to do, but it will all be within the framework of serving the state. Even within this, the largest compartment of them all, there are any number of sub-compartments. A scientist who, at the age of 40, feels he has run out of steam is not going to find the administrative or executive branches waiting with open arms for him, regardless of the undoubted variety of experience he might bring to a new job. Not, it must be admitted, that civil servants show all that much interest in changing their environment; a recent scheme to encourage temporary moves between the service and industry and academe, after two or three years of very modest success, is now in abeyance.

Because the civil service has built up great bodies of specialist expertise (some excellent, some not) within its walls, it feels relatively little need to share its problems with others or to draw in the outsider for a period to give a new perspective. This then leaves academics, for instance, ill-informed on many questions of broad national or international interest and inclined to withdraw even further into their laboratories, common rooms and learned societies. Not of course, that academics can be depicted as entirely innocent of their own compartmentalisations, both to keep the outside world at bay and to maintain fine distinctions between disciplines.

The end result of all this is by no means that Britain lacks experts in almost every subject under the sun, but that broader perception is often lacking. And surely scientists, as well as anyone, know how important a multidisciplinary approach is in the advancement of knowledge and understanding.

Of course, mobility must not be preached for its own sake. There are many who are at their happiest and most productive when pursuing their own specialisation, and these would profit little, and be much irritated by an ethos in which they were viewed with suspicion for sticking to what they do most effectively. On the other hand there are equally many who would profit greatly from the chance to diversify their career.

No single grand gesture will shake Britain out of its present over-specialised character. Rather a variety of smaller things have to be done: attitudes must change, terms of employment must be revised, even the penalty that the mobile suffer in their pension must be looked at. And there certainly ought to be more serious discussions about developing new institutions which could effectively span the public and private sector, looking at major policy problems but from an outsider's point of view. □

US boosts basic science, but overall R & D drops

Defence, energy and space head for large increases in basic research funding in President Carter's 1980 budget.

David Dickson reports

"The budget for fiscal year 1980 is lean and austere". The words that President Carter chose to open his budget message to Congress on Monday apply directly to proposed funding levels for research and development—but in a time of financial conservatism basic science has done relatively well, being scheduled for a real growth of 20% above the expected level of inflation.

The overall size of the R&D budget reflects the President's choice of reduced public spending as a major weapon to fight inflation. Thus total R & D obligations are planned to rise only 4.1% to $30.6 billion, the smallest increase in the R & D budget since the Nixon era.

Given this general constraint, the main issue that has occupied government agencies in preparing their budget requests has been to decide how the money should be distributed along the R & D spectrum And it seems to have been generally accepted that optimal long-term results can be achieved by shifting support for 'development' projects back to the basic research end (a trend initiated last year, though dealt with unsympathetically by Congress).

The net result of this is a projected increase of 9% in the funds available for the support of basic science, about 2% above the expected level of inflation, with increases of twice this amount for some agencies, such as the departments of energy and defence.

Those projects to have suffered are the ones which the administration feels have reached a stage at which they could be taken up in other ways—if at all. There will, for example, be a reduction in support for coal conversion technologies and hydroelectric and solar heating R & D, although more money for basic research in solar energy.

One agency to have done well in the budget review is the Department of Defense, which accounts for about 45% of federal support for R & D The department is scheduled to receive an increase of 7% in its total R & D funds to $13.8 billion, and within this an increase in basic research of 17% from $373 to $436 million.

The department's research money, whose growth reflects a general growth in defence spending that characterises the whole budget, will be used to support projects such as the MX intercontinental ballistic missile and new research on very high speed integrated circuits. There will also be more defence money spent in university research departments.

An even greater increase—18.8%—is scheduled for basic research supported by the National Aeronautics and Space Administration. However, here the figure is slightly deceptive, since much of this will be required to

Space scientists disappointed: The budget for the National Aeronautics and Space Administration does not contain any major new mission starts. It comes as a disappointment to space scientists, who had hoped that at least some of their proposals might have escaped the cuts. Top of the list of projects which will not receive funding are plans to send an orbiting imaging radar to Venus—particularly important in view of the failure of some of the equipment on board the current Pioneer missions—and plans for a gamma ray observatory.

Other casualties include a national oceangraphic satellite system, and plans to place a block order for a number of multi-mission satellites (mms). These will now be ordered individually, which could increase the costs of proposed British mms. Although these and other new projects were included in a $4 9 billion request which NASA put to the Office of Management and Budget, they were all discounted to ensure that general budget constraints and increased shuttle costs did not cut into existing programmes.

10% more for university research: The total federal R&D outlays to universities and colleges is planned to increase by 10% in 1980, considerably above the overall increase in R&D funding. The largest increase will be in research funds from the Department of Defense, scheduled to grow on campuses by 13% to a total of $359 million. Other major increases are planned for DHEW (11.9%) and the National Science Foundation (12.1%). In the proposed budget for the National Institutes of Health, funds for research projects awarded on a competitive basis are planned to fall from $494 million to $279 million, while non-competitive funds are scheduled to increase by approximately the same amount.

Boost for nuclear physics: After several years of relative neglect, there is some good news for nuclear physics, and government officials say that the increased support should continue "for some years to come". Funds have been made available in the Department of Energy's budget for a major heavy-ion facility at Michigan State University; an energy doubling electron beam recirculator at the Bates Linear Accelerator of the Massachusetts Institute of Technology; and an experiment staging area at the Anderson Meson Facility of the Los Alamos Scientific Laboratory. The expansion in nuclear physics has been partly made possible by the near completion of the Cornell storage ring, funded by the National Science Foundation. Construction of the Michigan facility is to be transferred from the NSF to the Department of Energy, although the foundation will continue to cover research costs.

Research on environmental hazards: As a reflection of the growing importance of scientific findings in the control and regulations of environmental hazards, the administration is proposing to make a major increase in the research budget of the Environmental Protection Agency. Obligations for R&D will increase from $400 million to $436 million, with a 16% increase in longer term research.

NSF instrumentation grants up 50%: The National Science Foundation is planning a 50% increase in its allocation of funds for research instrumentation and equipment, a source of growing concern in recent years and one of the areas in which universities and research institutions have been hit hardest by inflation. Announcing this increase, NSF director Dr Richard C. Atkinson said: "There is no question that we have been lagging behind in equipping our laboratories". In many Western European countries, the proportion of the research budget devoted to instrumentation was almost three times as high as in the US. The increase will raise the funds for instrumentation from $54.4 million in the current fiscal year to $81 6 million. The extra money is contained in a 12.4% increase in NSF funds for research in physiology and cellular and molecular biology, and a 15.4% increase for environmental biology.

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Carter: fighting inflation with spending cuts

support projects that have already been initiated—such as the Jupiter Galileo mission—but are now moving into an expensive phase.

Another burden on NASA's budget is the increased costs of the Space Shuttle, for which the agency is already having to approach Congress for an extra $185 million as a result of delays caused by engine test failures. The net result is that although there will be a 20% increase in NASA's support for space science and applications, there is no provision for any new programmes.

At the Department of Energy, which is having to bear much of the cutback in federal spending, there is said to have been considerable dispute over which end of the R & D spectrum should bear the brunt of the cuts. In the end demonstration projects were the ones to suffer. Within an overall R & D budget that will remain virtually constant in 1980 at $4.6 billion, there will be a 17% increase in support for basic science, sufficient to ensure continued support of high energy physics at current levels, as well as a boost for nuclear physics and solar energy research.

As far as the National Science Foundation is concerned, the administration has asked for a relatively modest overall increase in funding of 8.4%, bringing the agency's budget to $11.006 billion. Basic research is scheduled for an 11.8% increase, with large increases in chemistry, the earth sciences and environmental biology, and particular emphasis on upgrading equipment. Unlike previous years, no great increase is suggested for NSF's applied science activities. The budget for "problem-focused research applications" is to fall by 11.6%.

Projected figures for the Department of Health, Education and Welfare are a somewhat unreliable guide to what will actually be spent, since traditionally they are raised substantially by Congress. With this in mind, the administration has requested a budget for the National Institutes of Health of $1.4 billion, less than 1% higher than the current year.

Commenting on the overall budget figures, Dr Frank Press, Director of the Office of Science and Technology, said that "in general we feel that R & D has received good treatment. And when you add the basic research initiatives, we feel this is a very strong research budget."

Dr Press said that President Carter's initiative in increasing funds for basic research had become a model for other countries spurring similar increases, for example, in France, Britain, and West Germany. "It is nice to see that the US research dollar has leverage of this kind", Dr Press said. ☐

Sun shines on solar energy: Research on solar energy, a proposed cut in which last year brought a storm of protest from the environmental movement, is planned to receive an increase in funding of 24%. Longer term solar-related technology development and applied research will grow by 40% in 1980. In particular, the Department of Energy's budget request includes funds for the construction of a new 300-acre research facility at the Solar Energy Research Institute in Golden, Colorado.

Administration tries again on competitive agricultural research grants: The US Department of Agriculture is once more proposing a major increase in funding for competitive research grants, a proposal which has come under heavy fire in Congress for the last two years, during which such increase have been considerably cut back. In its proposed 1980 budget, the President has asked for a total of $30 million, two-thirds of this for basic research on the efficiency of food production, and the rest for the improvement of human nutrition. One major initiative in agricultural research is a major inter-agency effort to assess the value of space remote sensing data in obtaining early estimates of the effect of natural disasters on crop conditions, and improving world-wide agricultural production forecasting. Total funding for these projects, which will involve the departments of Agriculture, Commerce and Interior, as well as NASA and AID, is estimated to increase from $18 million in 1979 to $29 million in 1980.

No funds for Clinch River or nuclear reprocessing: The Administration is sticking to its previous position on two hot political issues in the nuclear field by requesting no funds for either continuation of the liquid metal fast breeder reactor at Clinch River in Tennessee or for nuclear fuel reprocessing. Although $504 million is being requested for LMFBR research and development, indicating a continued commitment to the fast breeder, the administration repeats its position that the proposed Clinch River demonstration project "is both premature and uneconomical".

Research on nuclear fuel reprocessing is proposed for termination in 1980, in line with President Carter's non-proliferation policy. In its place, emphasis will be placed on "once through" nuclear fuel cycle that will be used in conjunction with improved and more efficient light water nuclear power plants. Research funds for the Department of Energy provide additional support for the examination of alternative geological sites for a deep nuclear waste depository, and for developing new waste forms that could be used for the disposal of high level defense wastes. President Carter has also announced that he plans to introduce a "spent nuclear fuel Act" which will authorise the department to accept spent nuclear fuel for storage and disposal in return for a one-time charge.

Major effort in microelectronics: Three federal agencies—the Department of Defense, the National Science Foundation, and the National Bureau of Standards—are proposing to carry out complementary efforts in the field of microelectronics and submicron science and technology in 1980, involving a total budget of $41 million. At the NSF, efforts will be concentrated on fundamental research into the properties of microstructures, aimed at supporting a number of applications in electronics. The foundation will also provide training for scientists in this field, where a shortage of graduates is claimed by some to be holding back the efforts of private industry. The Defense Department is planning a major expansion of its research into very high speed integrated circuits, despite a major split in the defence industry over whether or not the technology already exists to meet the department's needs.

President halts child research building: Permission to construct a $37 million child health research facility at the National Institutes of Health has been withdrawn. It was considered low priority in a time of budget stringencies.

New US agency to focus research aspect of development aid

David Dickson reports from Washington on President Carter's proposal for a foundation for International Technological Cooperation

One of the relatively few new initiatives in the budget recommendations submitted by President Carter to the US Congress last Monday was the proposal to establish, under the foreign aid appropriations, a Foundation for International Technological Cooperation (FITC).

The foundation is intended to act as the focus for the research and development component of the US development assistance effort. It is presently being conceived as one element in a proposed international development cooperation administration, which would also include existing bodies such as the peace corps and the Agency for International Development (AID).

Funding for the FITC would be about $100 million for the first year of full operation, beginning 1 October 1979, although only a relatively small amount of this—about $25 million—would be new money, the majority of support resulting from the transfer of research projects currently carried out by AID. Eventually it is hoped that the foundations will have an annual budget of three to four hundred million dollars, the same order of magnitude as the National Science Foundation.

Plans for the foundation already figure prominently in the US national paper for the United Nations Conference on Science and Technology for Development (UNCSTD). However, criticisms of various aspects, such as the degree of autonomy which FITC will—or should—be able to exercise from existing aid programmes, and from overt US interests, indicate that the administration may face an uphill task in getting its plans accepted.

The proposed foundation was announced last March in a speech by President Carter in Caracas, Venezuela. It is largely the brain child of Dr Frank Press, director for the President's Office of science and technology policy, and reflects the same type of approach to R&D planning that he has injected into areas of domestic policy.

A planning office, reporting directly both to Dr Press and to Mr John G. Gilligan, administrator of AID, was established last summer. It is directed by Dr Ralph Schmuckler, previously dean of international studies and programmes at Michigan State University, and chairman of AID's research advisory board.

According to Dr Schmuckler, the foundation will have two main objectives, first, to help strengthen the scientific and technical problem-solving capacities of developing nations; and second to focus attention of US scientific resources on problems of concern to developing countries.

Although precise details are yet to be worked out, FITC is expected to operate by identifying various "problem-areas"—in contrast to the country-by-country programming of bilateral aid programmes—and then to work out which are the crucial problems in these areas.

"The programming process will be a bit like a funnel. At the top we will have all types of state-of-the-art analyses, with participation by agencies already involved, by developing country representatives, and by scientists. We would then move from a broad sweep to an increasingly narrow focus on particularly important goals that could be realised over a period of five to ten years", Dr Schmuckler said.

To illustrate the type of research that might result from this process, he cites work being supported by AID on the possible production of a vaccine against malaria, "A good example of an analysed, research problem dealt with initially in a very low-cost way, but resulting in some scientific breakthroughs that offer the possibility of a solution to a major health problem".

Besides research itself, however, an equally important part of the foundation's activities will involve helping to build research infrastructures in developing countries—for example by assisting in the training of scientists and technologists through courses and seminars, and occasionally providing short-term financial support for local research initiatives.

Above: protection against malaria. The disease disables 30–40% of the working population where the disease is rife. Research into a vaccine is "a good example" of the work FITC might undertake.

"We aim to spend the majority of our money in the developing countries themselves—a rough estimate is 75%", says Dr Schmuckler. "The rest will go to US universities and research institutions, but only to support work which is directly tied to activities overseas".

And to ensure what he calls a "real live audience" for the foundation's efforts, roughly one-third of FITC's advisory council—which will be responsible for recommending areas for study to the foundation's director—will come from developing countries.

Legislation to set up the foundation is soon to be presented to Congress as part of an amendment to existing foreign aid legislation. However, there are already signs that various criticisms of the current plan could lead to a tough political battle.

One criticism, for example, is that the foundation will be so closely linked to existing agencies such as AID that it will not have sufficient autonomy to apply an objective analysis to third world research needs (although others point out that it may be necessary to emphasise US interests to get the FITC accepted by Congress).

"The basic ideas is a very good one—but I am not very happy about the way that things are working out, particularly since the foundation seems to be becoming a subsidiary of AID almost as soon as it is born", says Professor Michael J. Moravscik, professor of physics at the University of Oregon, and an active member of the recently-formed Council on Science and Technology for Development.

Professor Moravscik and others are now putting their support behind an alternative proposal suggested by Senator Adlai Stevenson for a foundation for international scientific and technological cooperation, an agency similar in concept to the FITC, but having the power of autonomy enjoyed by the National Science Foundation.

A bill proposing such a FISTC is to be introduced into Congress by Senator Stevenson within the next few weeks. Attractive as the greater autonomy may be to some, however, its chances of success against the administration's FITC proposal, in a Congress increasingly concerned about public accountability for the use of federal funds, remains uncertain. □

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Cadmium puts UK village at risk

Cadmium, a light metal mined with with zinc, has been discovered in abnormally high amounts in stream sediment in the village of Shipham, the site of a zinc mine abandoned two hundred years ago. The Department of the Environment (DOE) has warned the villagers not to eat local produce and not to smoke and has initiated a £70,000 investigation to study the effects on health.

Levels of cadmium found in the soil varied from 11 ppm to 998 ppm. The accepted safe level is less than 1 ppm. Serious cadmium poisoning occurred in Japan from 1962 to 1968 caused by levels of cadmium in rice paddies of 1–50 ppm although there were other contributing factors in Japan that are not present in Shipham—such as dissolved cadmium in water.

Shipham water has been tested and is "absolutely safe" said Mr Denis Howell (a minister in the Department of Environment). "There is cause for concern but not for alarm. It is in nobody's interest to exaggerate the alarm" said Howell. Local residents have known for years that vegetables grow yellow in certain locations, and there is a local ailment—called "Shipham tummy"—which could be a sign of chronic cadmium exposure. Long term effects are poorly known but symptoms of cadmium poisoning are diarrhoea, vomiting, stomach pains and choking.

The high concentrations were discovered in the course of an Imperial College geochemical reconnaissance of England and Wales begun in 1967. The study took 50,000 stream sediments from tributary drainage over the whole country during a ten week period in 1969. A complementary sampling of soil and rock was undertaken in Shipham to test for the presence of elements in areas of poor drainage. The high cadmium count found in this survey was followed up by sampling garden soil in conjunction with researchers from the Westminster Hospital Medical School in Spring 1978. These results were released to the DOE last August.

Dr Ian Thornton, Geochemistry Department at Imperial College and participant in the original study says that Shipham is probably unique. Calamine, $ZnCO_3$, was mined in Shipham whereas in other mines it was galena, zinc sulphide.

Joe Schwartz

Biochemist challenges redundancy

An industrial tribunal currently underway in Manchester raises new questions on the power of granting agencies to make scientists redundant. Dr David Pillinger, FRIC, 39, a biochemist employed at the Christie Hospital in Withington near Manchester, is the first senior staff member of the National Health Service to be made redundant since 1948. Pillinger's case, brought to the tribunal with the aid of ASTMS, squarely confronts a complicated legal issue affecting thousands of scientific workers in the UK; who is their employer ? Is it the agency that provides the money or the institution that does the hiring?

Pillinger was hired in 1962 by the Manchester Area Health Authority to do cancer research at the Christie Hospital. At that time there was no special category for research workers and Pillinger was engaged on the same basis as the clinical biochemists and physicists, with a permanent NHS contract, the funding coming from the British Empire Cancer Campaign In this arrangement the Health Authority provides a permanent contract even though it does not provide the funds, with the understanding that the contract will be underwritten by the Authority should outside money dry up.

In 1972, Professor L. G. Lajtha, Director of Research in the Christie Hospital's Patterson Laboratory, attempted to bring all research workers under MRC short term contracts of three to five year duration. Pillinger refused to sign such a contract but eventually was persuaded in 1973 to take a post of unlimited duration on a block grant, a form of funding that covers several different projects. This was a hybrid contract in which the employer was still the Health Authority but the money and pay scales were set by the MRC. Says Pillinger, "There is no doubt, nor has it ever been questioned, that my employer is the Area Health Authority."

Nevertheless in June 1978, Pillinger was told by Lajtha that he was to be made redundant. In a letter from the MRC Lajtha was told that there was to be no reduction in funding for Pillinger's chemotherapy project but that the MRC did not feel "that Dr Pillinger should continue to be supported even though a senior scientist is needed" Pillinger pressed Lajtha for an explanation and in notes submitted to the tribunal quotes Lajtha as saying, "I doubt if we will ever be told, since the MRC is an autonomous body. It does not have to give account to anybody for any of the decisions they take."

The Christie Hospital administration is supporting Pillinger. Mr D. C. Critchley, former Sector Administrator, was called by Pillinger's counsel, Ms Janet Smith, to testify last Monday Lajtha, however, is critical of the hospital administration. Calling Pillinger's case a "purely administrative problem" Lajtha feels that Pillinger's original contract was a case of "administrative irresponsibility" and that the old management should have made it clear that a scientists's employment must depend on the ebb and flow of grant money. Lajtha says that the MRC project grant renewal did not permit him to employ a Senior Grade Scientist but only a Grade 2 Scientist (equivalent to a Lecturer). When asked why he did not make representations on Pillinger's behalf to the MRC Lajtha said that he had already presented the case to the MRC and that they were "fully in the picture".

Behind the official language of dismissal and tribunal, however, lies a story of research conflict and scientific disagreement. In 1969 Pillinger returned from a two year stint at Columbia University in New York where he worked with Ernest Borek on tRNA modification by methylation. Pillinger began work on this line at the Christie Hospital and produced 12 publications in three years. In 1972 this project was terminated by the MRC. Lajtha requested that Pillinger start work on a model of cancer cell proliferation in which tissues were hypothesised to produce proliferation inhibitors Pillinger's job was to isolate the putative inhibitors called chalones. The technique used cell extracts in water and the fraction of MW of around 1000 was observed to inhibit cell division. Pillinger was critical of this work and thinks that the results were artefacts (The cell division assay used radioactive thymidine as a marker and Pillinger felt that the cell extract fraction found to inhibit division probably contains thymidine so that a decrease in thymidine uptake was an artefact of the preparation.) In 1974 Pillinger transferred to other work on cancer chemotherapy with Brian Fox, Head of Experimental Chemotherapy. It was on this project grant that the MRC found that Pillinger's services were no longer necessary.

Joe Schwartz

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UN desert unit to concentrate on national projects

DISILLUSION with transnational programmes to halt the growth of the world's deserts is causing the United Nations Environment Programme (UNEP) to turn to specific national projects, the new director of UNEP's desert unit said last week.

The director, Jens Høgel, said that the criticisms of Professor Mohamed Kassas (reported in *Nature* last week, p167) were "a long step ahead" from the original political naïvity of the 1977 United Nations Conference on Desertification (UNCOD), which proposed six giant transnational projects based more on scientific optimism than political reality. Professor Kassas was in fact a major force behind UNCOD.

UNCOD passed the six projects on to UNEP, which then had the task of implementing them. Details of the projects—which include matters such as the management of the massive North East African fossil water aquifer—are still being drawn up, but, says Høgel, "it is better to put our eggs in national baskets".

One such plan is for the establishment of new industries in arid zones to attract populations back on to desert margins. These would bring water with them and help to stabilise desert movement. Nine countries—Afghanistan, Algeria, India, Iraq, Mali, Mexico, Peru, Spain and Tanzania—are submitting project proposals to a meeting which will take place in Moscow from 5–8 February. The USSR will provide the necessary funding.

Nevertheless national progress "is still going a bit too slow". And while many rich northern states "have indicated very clearly that they are interested" in supporting UNEP's desert work, no money will be forthcoming from them until specific projects are agreed.

"But there is no reason for despair" says Høgel. It took 30 years from the recognition of the problems of development around the time of the second world war before effective action began to be possible through the United Nations Development Programme (UNDP) and so on. "We have just recognised desertification (at UNCOD) in October 1977. But it won't take three decades now; we have UNEP, a new will to succeed, and a tremendous pool of international expertise to call on."

Nevertheless there is a great deal of inertia to overcome. The independent Club du Sahel, funded mostly by the US and France, was created in 1975, "but when I left the Sahel four months ago there still wasn't any action".

Høgel is adamant that the UN organisations have not been "sluggish and inefficient" as charged by Professor Kassas. "We are at the top of the scale compared with most bilateral agencies, and what is more you can't lose the United Nation's goodwill." On the other hand, Høgel can see bilateral agencies imperialistically forcing their solutions onto the recipient countries. "This leads to reticence, the resulting institutions won't function, and it will breed ill will. And the recipient countries hold their best people back".

The approach of UN agencies, like UNEP's desert unit for example, is to encourage the individual countries to create their own projects, "so there is no question of imperialism". Furthermore the UN can call on expertise internationally, whereas bilateral agencies are limited to the donor country.

In the Sahel, however, Høgel recognises the enormous resources that the Club du Sahel can call on—some $2,000 million compared with $60 million in UNEP's Sahelian office—"and the beneficiaries are the same". The Sahelian population is the worst hit and also the poorest desertification area in the world.

Høgel: supporter of national projects

The desert unit at UNEP is still very small. UNCOD recommended a professional staff of ten, and some think it should be larger, but so far Høgel has himself and five others. Previously Høgel was a development manager with UNDP in Upper Volta; he is a 46-year-old Dane with wide experience in the third world. To assist him he has a Soviet professor in soil science, a Sudanese ex-vice-minister of agriculture (and a doctor of veterinary medicine), an Afghani programme officer, and a Belgian meteorologist. The latest recruit is a Malian ex-nomad turned sociologist of nomadic populations.

The unit's work covers the six transnational projects, establishing new national projects, organising national courses on the most basic desert management, and map-making. The original UNCOD map has been widely criticised as impractical, on too small a scale, and lacking clear definitions of the desertification process. A new one at 50 to 100 times the scale (1:1 million) is being drawn up by Professor Harold Dregne at Texas Technical University, but it will take two years and is already leading to a lot of disagreement on defining borders. "But the footwork needed to arrive at the map is very important" says Høgel. "At least we ought to know in detail where the danger regions are."

Robert Walgate

"He drinks one, then needs three more to forget about the cancer in the first."

German scientists detect carcinogens in beer

SCIENTISTS at the German Cancer Research Centre in Heidelberg have discovered traces of nitrosamines, one of the strongest known cancer inducing agents, in beers made in Germany, Britain and other European countries.

A two-year study of 158 beers financed by the German government, with support from the US National Cancer Institute, revealed traces of nitrosamines in 70% of the samples. The chemical, which is thought to be produced by contact with nitrous oxide when the beer's malt is dried, was found in much higher levels than in foodstuffs such as pickled meats, ham and sausage also included in the study.

Dark beers and stronger beers contained higher levels of nitrosamines than light beers such as lager and pilsner, according to the head of the research team, Professor Rudolf Preussman.

David Dickson

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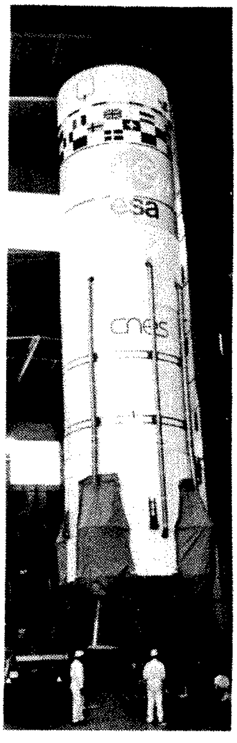
news in brief

British Association meets on UNCSTD: As preparations mount for the UN Conference on Science and Technology for Development (UNCSTD) in August, the British Association for the Advancement of Science is organising a one-day public symposium on March 8 to allow 'non-government bodies' a chance to express their opinions. Seven main speakers will put points of view ranging from that of the UN, from Guy B. Gresford, who is Deputy Secretary General UNCSTD, New York, to the 'trade union view' by Barry Sherman, Director of Research at ASTMS. Sir Ieuan Maddock, who will also chair the meeting, presents 'a British scientist's view', while the multinational companies will be covered by Mrs Doreen Wedderburn of Unilever and chairman of a CBI working group. Professor A. H. Bunting (Reading University) and Adrian Moyes of Oxfam are scheduled to present the reaction of the late-developing countries and the 'very poor' to science and technology; while Professor Robert A. Shaw, who holds the chemistry chair at Birkbeck College, London, will speak on the educational consequences of UNCSTD, in a talk that will undoubtedly range well beyond its title.

The BAAS seems confident that the meeting will be both lively and useful. With this line-up of speakers and the quality of contributions expected from the floor, a forum of this kind should be the best way of putting across the 'unbiased opinions'—in a BAAS phrase—of British scientists to the British UNCSTD delegation. (Further details from the symposium secretary, 01-734 6010 extension 377.)



Accident delays European Space Launcher: Development flights of the European Space Launcher, Ariane, have been delayed five months due to failure of a safety device on 28 November 1978. An ignition delay in the combustion chamber caused an excess of hydrogen to build up which resulted in a "violent ignition", according to ESA. The explosion caused extensive damage to the propulsion system requiring the launcher to be dismantled and refurbished. But initial fears that the stage or propulsion design might have been responsible were unfounded. An inquiry conducted by the project team found that a malfunction of a hydrogen flow monitor was responsible. The new dates for the first four development flights are: early November, 1979; early March, 1980; June, 1980; and October, 1980 (unchanged). The revised schedule permits the first operational flight to go ahead as scheduled in December 1980.

US scientists urge further intensive study of Mars and Venus: Mars, Venus and the Earth should receive the major focus in exploration of the inner solar system for the next decade, according to a report published recently by the National Research Council, the study section of the US National Academy of Sciences. "The comparative planetology of these bodies is a key to understanding of the formation of the earth, its atmosphere and oceans, and the physical and chemical conditions that lead to the origin and evolution of life", the report says, although it adds that the atmosphere-free terrestrial planets, namely Mercury and the Moon, are "complementary bodies of high scientific interest".

As for further missions to Mars and Venus following the Viking missions of 1977 and the current Pioneer missions, the report says that studies should be initiated to develop the special technology needed for the return of samples, as well as *in situ* experiments. In an "overview" section of the report, the committee says it is deeply disturbed by the near-term outlook for the US planetary programme, and that the struggle in 1978 to obtain approval of the Jupiter Orbiter Probe, after a three-year hiatus in planetary mission starts, was a reminder that a national policy for planetary exploration was still to be defined.

The report also says that in view of the lack of direct and substantial information on Soviet plans for planetary exploration, and because of the limited extent of past scientific exchanges, "it appears desirable to establish a much improved vehicle for discussion of US-USSR interests in planetary exploration".

US to assess environmental impact of foreign projects: All US federal agencies must in future prepare an assessment of the likely environmental impact of any new project to be carried out in a foreign country, following an executive order signed last week by President Carter in Washington. Projects that could be affected by the order include the construction of a nuclear reactor in the Philippines, which critics claim to be dangerously near an earthquake fault, and US participation in spraying Mexican marijuana fields with the herbicide paraquat.

The presidential order follows almost a decade of dispute over whether the National Environmental Protection Act of 1969, which requires environmental impact statements on federal projects within the US, was also intended to cover projects carried out overseas. The Council on Environmental Quality has always maintained that this was, indeed, the intention of the US Congress in passing the Act; in contrast, the US State Department has claimed that carrying out such an assessment would be a violation of the sovereign rights of other countries. Dr Charles Warren, chairman of the Council on Environmental Policy said that the order was a response to a growing worldwide concern that governments are undertaking major actions without enough consideration of the consequences.

Libyans deny A-bomb intentions: Libyan officials have denied charges by the US Federation of American Scientists that the country intends to acquire nuclear weapons. According to Mr Ahmed Shehati, head of the foreign affairs office of Libya's ruling People's Congress, Libya has "neither the time nor the potential" to acquire nuclear weapons itself, although it believes that the superpowers should curb any further transfers of nuclear-weapons technology to Israel, South Africa or Rhodesia. The charges were made after a trip to Libya two months ago by FAS secretary Dr Jeremy Stone.

Birmingham University challenges smallpox trial: Birmingham University has won leave to apply to the High Court to quash its prosecution over the smallpox case. The university claims the trial has been prejudiced by the premature release of the Shooter report on the incident.

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No crisis, but save energy, say Soviets

Vera Rich reports on Soviet energy in a year when nuclear generating capacity in the USSR is to increase by 50%.

DURING the past year, Soviet energy planners have issued a number of highlevel denials of anything approaching an energy crisis. Since the publication of the 1977 report of the US Central Intelligence Agency which predicted that the Soviet Union would become a net importer of energy by the late 1980s, considerable effort has been put into official denials.

And yet in 1976 a major publicity campaign was launched to convince the population of the necessity to save energy in all its forms. According to official policy, this does not imply an energy crisis—it is simply intended to make people aware of the need to use the natural resources of the country "rationally". The policy-makers insist that sufficient energy resources exist, and any temporary set-backs are due to the geographical and logistical problems of exploiting them or, alternatively, slackness in some vital part of the energy industry or its auxiliaries.

Such slackness, even at the highest levels, is not to be tolerated. At the end of December, the Minister of the Coal Industry of the Ukrainian USSR was dismissed on the grounds that his ministry had been unable to rectify the production lag in the coal industry. For some years coal occupied a less elevated place in Soviet planning than other forms of energy, but it now appears to have been re-emphasised. Prospecting for new coal deposits has been going on, not only in the unexplored regions of Siberia, but also in the European parts of the USSR. During 1978, two major new coalfields were discovered at Lisichansk and Bogdanovskoe in Ukraine, conveniently close to traditional centres of industry. It is hoped both will be fully productive by 1985.

Unfortunately, most major energy sources in the Soviet Union are not so conveniently placed. The main centres of population lie west of the Urals; the new oil and gas fields lie far to the east. There are clear indications that the exploitation of these deposits is proving far more costly, both in money and in time, than was originally expected.

In January 1978, a group of foreign journalists on an official tour of the west Siberian oil fields, 'accidentally' met Sergei Velikopol'skii, First Secretary of the Communist Party branch at the administrative headquarters of the Samotlor field, the largest oil field in the Soviet Union. It is true, he admitted, that the output of the Samotlor field will level off over the next seven or eight years, and will then decline, but this will be more than offset by bringing the east Siberian fields on-stream. The fact that production from Samotlor will peak earlier than expected is merely due to the extreme climatic conditions encountered in the east Siberian fields. In order to meet production plans, more oil has had to be taken from Samotlor than originally projected for the current planning period.

In spite of the apparently optimistic tone of this interview, Velikopol'skii's remarks seem to suggest delays in the exploitation of the east Siberian fields. Permafrost or swampy terrain hardly provide optimum drilling conditions —although in the latter case, there has been encouraging progress in the use of slant drilling from artificial islands. Transporting the oil westwards is, however, an even more costly business. According to Academician Mikhail Styrikovich, it costs about 1 million roubles (a rouble is about $1) to lay 1 km of pipeline in this terrain, while the construction of workers' settlements costs 50—100% more than the construction of similar settlements in developed areas.

Sometimes, indeed, the logistics of oil extraction become ludicrous. At the Urengoi gas deposit, not only did the base camp have no proper maintenance facilities for equipment, all fuel for motor vehicles and tractors had to be hauled some 2000-3000 km, so that the transport costs exceeded those of the fuel itself. Only after some considerable time was it realised that motor fuel could be produced on site from the gas itself, and an experimental unit, capable of producing some 35 tonnes of fuel every 24 hours was installed on site.

Although unwilling to admit to an energy crisis, the Soviet authorities do admit, under pressure, to having underestimated their production costs This January Moscow radio, replying to an article in *Le Monde* which censured recent increases in the price of Soviet oil to the Comecon block, admitted an additional investment cost of 7,500 million roubles, simply to keep up the current level of supplies to Comecon. And this, it claimed, was in spite of there being no transport cost, since the outlay on the "Friendship" pipeline had already been recovered.

Furthermore, comparison of the official figure for pipeline built during the five-year-plan 1971-75 (12,000 km) and the amount of large-diameter pipe bought from abroad (1,000 million tons—sufficient to build some 13-15,000 km of pipeline, depending on wall-thickness), it would appear that the pipelines were at that time built largely from imported pipes. (The situation may have changed recently, judging from complaints last year that certain Soviet factories charged with producing the required pipes had failed to do so, thus delaying pipe-laying operations). Add to this auxiliary technology purchased abroad—high capacity compressor stations (Rolls-Royce), pumps for water-flooding techniques (US), desulphurisation equipment (France)—and the expenditure of precious foreign currency reserves must be considerable.

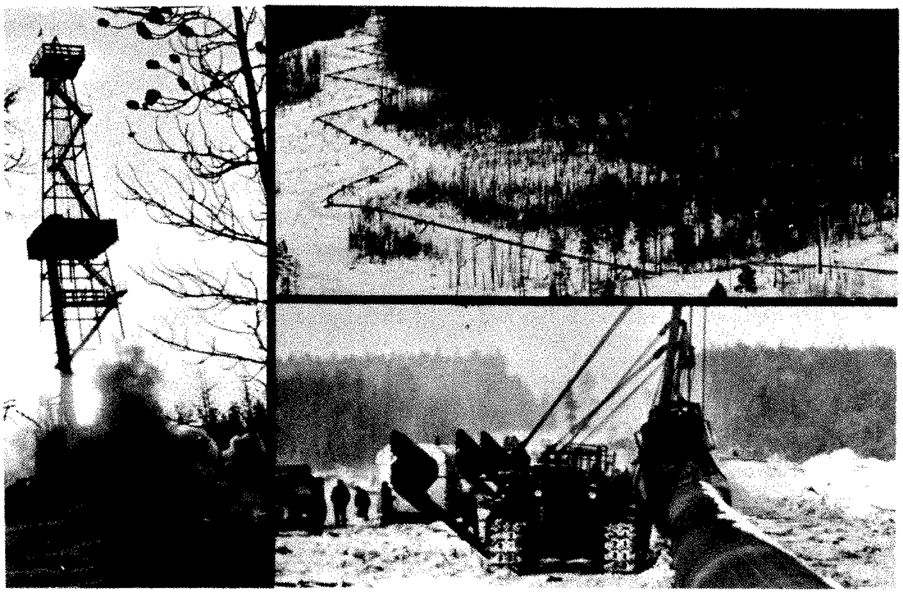
Even the Soviet Union's "prestige" position as major oil supplier to Comecon, has been falling since the sudden increase of oil prices to the bloc, in September 1976 (following pledges not to increase). While still paying lip-service to an integrated oil and gas policy for the Comecon bloc, a number of the individual countries are quietly pursuing their own alternative plans—East Germany the underground gasification of coal, Poland a country-wide survey for oil and gas resources, and Romania an off-shore drilling programme.

However, if the great pipelines, with their evocative names of "Friendship", "Brotherhood" and "Union" are more expensive as a means of integration than was first hoped, there still remains the new electricity link between Vinnitsya in Ukraine and the Comecon terminal at Albertirsa in Hungary. The Soviet Union has considerable plans for expanding generating capacity.

A unified power grid for the country is being completed, which would obviate any major differences in peak and non-peak hours. Since the Soviet Union is so wide east to west, there is virtually always a peak period going on somewhere in the country, to which electricity could be relayed from some less busy region. (The possibility of cryogenic superconductor transmission is seriously under consideration)

Speaking on "Power Workers' Day" last December, Petr S. Neporozhnii, the Minister of Power and Electrification, told *Pravda*, that in 1979, the generation of electric power is to be brought up to 1,265 GW, which would include an almost 50% increase in nuclear power generation.

The stress on the nuclear branch is not accidental In spite of the unified grid, it would clearly be more convenient to generate electricity close to where it is required. The major sources of fossil fuel for thermal power



Drilling in Siberia (left) is tough enough: then comes the problem of getting the gas and oil back to population centres.

stations, and the large rivers still not committed to their full hydroelectricity capacity lie far to the east—but a nuclear power station in the Soviet planners' opinion, can be built wherever required.

For the Soviet planners do not have to contend with an antinuclear lobby; even the dissidents seem to have accepted that nuclear power is an intrinsic good (unlike the Czech Chartists who recently demanded a referendum on the whole issue).

Repeatedly, official spokesmen stress that nuclear power stations are safe and do no damage to the environment. A book published in 1974 to celebrate 20 years of nuclear power generation did, indeed, contain a chapter on safety, but this only refers to radiation protection within the plant itself.

The Soviet planners seem convinced that no major accident can possibly occur. According to one US nuclear delegation, which visited the Soviet Union in 1970 "Reactor design is based on the assumption . . . that there is no credible accident corresponding to a severe accident excursus or total loss of coolant . . . The safety philosophy for light water reactors is similar to that for the LMFBR's in the USSR. For example, a double ended pipe break is considered incredible; therefore, no separate emergency core cooling systems, no containment buildings, no ice condensers, and no pressure suppresant chambers are provided".

With this somewhat casual attitude to safety, the Soviet Union is embarking on a major nuclear power programme. Last year new power sets were commissioned at the Leningrad, Novovoronezh, Chernobyl and Kursk power stations, and at present 13 nuclear power stations are under construction. Set capacity is to be increased from 440 MW to 1000 MW and there is even talk of sets of up to 2500 MW being feasible.

Most of the new stations are RBMK uranium-graphite boiling water reactors or water-cooled, water-moderated reactors. However, the present five-year plan commits the economy to the development and construction of fast-breeder reactors.

A pilot 12 MW fast breeder was constructed in Leningrad in 1968, and at Shevchenko, on the Caspian sea, a fast-breeder has been in operation since 1973, acting as the sole source of electricity and water desalination for the town. This was the reactor which in 1974 was observed by US surveillance satellites to have been damaged by an explosion although, according to official Soviet sources, their satellites failed to record anything amiss and the local population "did not even know that anything had happened". (Unofficial sources, however, reported considerable panic). Even when an off-the-record comment on the accident can be obtained, it is always presented as a small conventional explosion due to the contact of liquid sodium and steam in the heat exchanger—not properly speaking a nuclear event at all.

A second FBR being built at Beloyarsk was originally scheduled to be completed in 1973, but will probably be commissioned this year. Both these FBRs are uranium-based, but according to Mikhail Troyanov, Deputy Director of the Institute of Physics and Power Engineering "we see no difficulty in moving to plutonium. After 1990, breeders should be built in the USSR in large numbers".

Not only are the Soviet planners prepared to site power stations in centres of population; they are equally ready to use nuclear power to provide district heating, and as an industrial steam source. One such system is already operating at Bilibino on the Chukotka peninsular. Most commentators assume that such installations would be a combined source of electricity and heat, but mention has also been made of a special low-temperature, low-pressure water cooled reactor which could deliver 500 MW heat but no electricity, and which could possibly go into production at the beginning of the 1980s.

Against this background of expansion, there is a constant drive for more and more fuel economy. A number of less conventional energy sources are under investigation—windpowered generators are already being manufactured for use in the sheep-rearing areas of Moldavia and the far north, and the possibility of constructing solar power stations in the arid steppe is receiving serious attention. At the Institute of High-Temperature Physics, research is going forward (with US cooperation) on MHD generation as a means of utilising fossil fuels more efficiently.

The Far Eastern Science Centre is working on the possibility of using geothermal heat—in accordance with high level directives that they should devote their main attention to the problems and potentialities of their own area.

In spite of the expected advent of the supergrid, the possibility of pumped storage stations has not been overlooked, and the construction of such a station at Zagorsk was begun last September. And, since the fuel-saving campaign was inaugurated in 1976, (it was intensified in September 1978) there has been a constant discussion in the media of possible means of economising. These range from the domestic—one appeal on television to turn off unnecessary appliances is said to have saved 10 MW in a single hour—to new means of using fuel. The latter range from genuine savings—such as the saving of "more than 17%" of all thermal energy needs at the Kemerovo fertiliser works by using the heat from exothermic reactions, to advice about burning maizestalks or woodchippings, which seem more a symbolic gesture than a piece of practical economy.

Nor is the energy industry prepared to shoulder all the burden. One letter to *Pravda* from a manager in the Leningrad power industry suggested that it is not the power workers who should think of energy-saving, but the architects and designers. Their "window-mania" of the last few years, he claimed, has caused unnecessary increases of up to 60% in the cost of heating living and working space! □

correspondence

Short term contracts

Sir,—It is sad that three recent articles (23 November, page 310; 28 December, pages 743 and 745) on the difficulties facing research workers in universities have induced little dialogue in your correspondence columns. It has been our association's experience that there are at least two reasons for such a lack of response: (a) research scientists are often isolated in departments with a high percentage of tenured staff and think that protestation would have little or no effect; (b) many fear that if they do step out of line and "speak out" they will not only jeopardise their present job but will find it even harder to secure another. Presumably, it is because of this that the present appalling situation has been allowed to develop. We therefore would like to appeal to all researchers to start thinking positively, join a union and become active in it, join our association (if appropriate), or form an association and write to your MP, asking for an inquiry. We demand to have a career structure in research with some prospect of security so that a PhD will come to be regarded again as an attribute and not a liability!

Arleen Unger

ARMS, London, UK.

Sollas and the Sherborne bone

Sir,—I would like to correct the record of the Sherborne engraved bone controversy, unfortunately misrepresented in Dr L. B. Halstead's account of the late Professor Douglas's tape-recording linking Sollas with the Piltdown hoax (2 November, page 11). Douglas's story reveals nothing fresh except his own assumption that Sollas kept quiet about a 'hoax' at Sherborne School (Dorset) so that Smith Woodward might make a fool of himself; the rest is a tendentiously garbled summary of Bayzand's evidence against the bone in *Nature* 117, 233; 1926, confounded with facts stated by Smith Woodward himself (*Nature*, 117, 86; 1926).

Dr K. P. Oakley established in 1957 that the piece of bone was ancient and consistent with an Upper Pleistocene date. It is clear to the naked eye that it is semi-fossilised, as Smith Woodward claimed in his reply (*Nature* 117, 86) to Sollas's denunciation in *Ancient Hunters* (1924). Sollas (*Nature* 117, 233) was nonplussed by this claim and attempted to parry it with an ignorant remark betraying that he had never seen the object. Neither had Bayzand! Had he done so he could not have left Sollas in ignorance of a fact so vital to any assessment of a schoolboy hoax, or failed to investigate an ostensible fake of extraordinary ingenuity, as his wholly imprecise charge shows It is impossible to resist the conclusion that Bayzand heard a story, true or false, and simply did nothing at all—until 1914, as he himself tells us, when Smith Woodward's paper appeared (*Q. Jl. Geol. Soc.*, 70, 100–2).

It is generally supposed that Sollas and Bayzand were left with the last word, for Douglas evidently believed them, as did the late Joseph Fowler of Sherborne, whose exhaustive but uncompleted research led to my own interest in the affair. There was, however, a third letter in *Nature* (117, 341–2; 1926) only weeks later, from the supposed hoax victim, R. E. Steel, who began by apologising for Bayzand's having been misled by senior boys who had been unable to credit such a find by two 14-year-olds, Arnaldo Cortesi and Philip Grove, in their first weeks at the school. His account of the circumstances of discovery, corroborated by a former pupil who had recognised the character of the engraving and prevented Cortesi from throwing it into the day-room fire, and the aptness of the alleged site (thereupon efficiently investigated by Steel), are entitled to respect.

The question remains whether there was anything more than school rumour in the story Bayzand treated so casually but with such disastrous consequences for a potentially rare and interesting find. Was the engraving nevertheless faked, and if so, whence came the material and expertise? One can imagine Douglas's answer—one that would at least make sense of his otherwise forced analogy between Sherborne and Piltdown—but I hope shortly to deal more fully, in *Antiquity*, with this and other aspects of this curious tale.

R. A. H. Farrar

Royal Commission on Historical Monuments,
London, UK.

Seveso

Sir,—Professor Garattini and Dr Manara of the Istituto di Ricerche Farmacologiche Mario Negri, Milan, have accused me of underestimating the possible damage to human health caused by the Seveso accident (7 December, page 556).

I took on the assessment of the damage to health caused by the accident because I had been personally involved from the beginning in protection and prevention measures for the injured and for those still exposed.

I played an important role in the decision to evacuate the population of Seveso. This has been stated in several books on Seveso and in official documents in my possession. I never expected that readers of my papers would accept on sight my assessment of the consequences of the accident on the health of the population, and I have therefore always based my reports and conclusions on documents[1–10] which cannot be accused of prejudice in my favour. The great majority of these authors are either members of the Epidemiological Medical Commission of the Lombardy Region in Milan (of which Professor Garattini has been a member since its beginning) with responsibility for the health surveillance of the population, or of the Advisory Scientific Committee to the Italian Government in Rome for the Seveso accident (Commissione Cimino).

They have been (and still are) confronted with one of the most difficult and tricky problems in medicine. They have mastered the emergency with courage, skill and competence. The dermatological screenings carried out by them on 32,000 schoolchildren have no precedent in the history of clinical toxicology. I trust their reports to be accurate, meticulous and unbiased.

Nevertheless I may have overlooked or missed data available to Professor Garattini and Dr Manara which would prove me wrong. I therefore ask Professor Garattini and Dr Manara to make any such data known immediately, because only then (and not by saying that I am biased) will it be possible to evaluate the damage.

About 5.000 people are still exposed to levels of TCDD which, while admittedly lower than those of past cases of occupational exposure, could represent a potential risk. If my conclusions are wrong, we have a moral obligation to take protective measures immediately to prevent any further damage to this population.

G. Reggiani

Hoffmann-La Roche

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3 Fara, G M *ibid*
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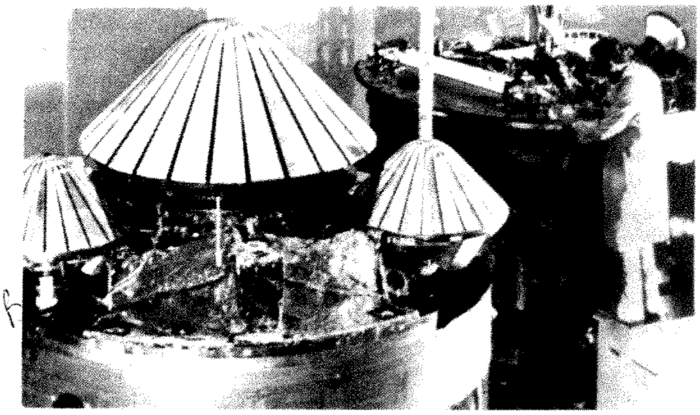
7. Pocchiari, F. Human health effects from accidental release of TCDD at Seveso (Italy) International conference on health effects of halogenated aromatic hydrocarbons New York Academy of Sciences, 24–27 June 1978
8 Puccinelli, V. *et al*. Skin disorders in the Seveso area after the accident at the Icmesa plant Reports to the Chloracne Panel of the Commission of the European Communities Directorate for Employment and Social Affairs, Division of Health and Safety. Milan, 11–12 July 1977, January and May 1978
9 Dioguardi, N. Critical considerations two years after the accidental pollution of Seveso. Press Seminar "July 1976–July 1978" Analysis and evaluation of the consequences of pollution by TCDD and of the public policies adopted two years after the Icmesa disaster 2 July 1978.
10. Report of the Italian Parliamentary Commission of Inquiry Chapter 2, pp 145-181 The after effects of the incident of 10 July, 1976 on the health of the population exposed to the risk of contamination. Rome, July 1978.

news and views

Epithelial cancer, differentiation, and vitamin A

from Brigid Hogan



MORE than 90% of people who die of cancer are killed by metastasising tumours of epithelial tissues. These malignancies do not develop rapidly, but evolve over one or two decades through stages in which there is increasing disorganisation of the epithelial layer and its association with the underlying stroma. Studying this latent period is like piecing together a complex and tantalising jigsaw puzzle; complex, because the pieces represent all the agents such as hormones, growth factors and tumour promoters which affect the proliferation, organisation and differentiation of epithelial cells; tantalising, because we obviously still lack many of the vital pieces needed to complete the picture and so perhaps learn how to intervene therapeutically between the initiating event and the final malignant state. One piece of the puzzle are the retinoids, a class of compounds based on vitamin A which for a long time has been known to have profound effects on many epithelial tissues.

Retinoids in organ cultures

The effect of retinoids on epithelial cells has been studied for many years. In young animals vitamin A deficiency results in the transformation of simple, ciliated, mucous-secreting epithelia (such as those found in the respiratory and genital tracts) into multilayered, non-secretory keratinised epithelia, a process known as squamous metaplasia. The change can be prevented (or reversed) by adding retinoids at concentrations down to about 10^{-10} M (see Clamon *et al.* *Nature* **250**, 64; 1974, for the standard assay using pieces of trachea from vitamin A-deficient hamsters). With higher concentrations of retinoids the tracheal cartilage starts to break down and proteases and hydrolases released from the chondrocytes begin to digest away the matrix. This response is usually considered to be simply the result of the detergent-like action of high concentrations of polar retinoids on the cell membrane, but others might argue that it reflects a physiological response of chondrocytes, important in tissue remodelling during early development. Of the natural retinoids, retinoic acid is the most effective in reversing squamous metaplasia and can be given in much higher doses as it is not stored in the liver.

Almost nothing is known about the way in which retinoids mediate their effect on epithelia. For example, they could act on the epithelial cells directly, or indirectly by provoking signals from the underlying stromal cells. It is possible that, like the steroids, the retinoids have to enter the cell to be effective; several cytoplasmic retinoid-binding proteins have been isolated from a variety of whole organs, and the affinity of one of these proteins for different analogues correlates with their effectiveness in preventing squamous metaplasia in organ cultures (Chytil & Ong *Nature* **260**, 49–51; 1976).

Retinoids and carcinogenesis *in vivo*

One of the most exciting new ideas in the study of retinoids is the possibility that they can intervene in the process of epithelial carcinogenesis *in vivo*. Several months after giving a dose of carcinogen to experimental animals, the target epithelium shows areas of disorganisation, ranging from hyperplasia (increased mitotic rate of the basal cells), squamous metaplasia and papillomas, to increasingly invasive carcinomas. A number of groups have reported that feeding the animals high doses of retinoids after the carcinogen treatment reduces the severity and often the incidence of the lesions in a variety of epithelial sites (for example, bladder, lung and trachea) (for reference, see Sporn *et al.* *Fed. Proc.* **35**, 1332; 1976). In these experimental systems, retinoic acid has proved to be more effective than other natural vitamin A derivatives. (Some workers have reported that retinoids have no effect on tumour incidence, but Sporn has argued that this is because retinol derivatives that can be removed from the circulation and stored in the liver were used). The action of retinoids therefore seems to be exactly opposite to the effect of tumour promoters—naturally occurring chemicals like the phorbol esters which do not themselves cause cancer but increase the rate at which tumours appear in response to carcinogen treatment (see *News and Views* **270**, 659; 1977). The retinoids, in other words, seem to be acting as 'anti-promotors'. But before readers run off to order mega-doses of retinoic acid, it must be stressed that the story is by no means simple, and there are reports that retinoids can enhance tumour growth, although in these cases the vitamin derivative was applied in high and possibly irritant doses to the skin rather than included in the diet (Polliack & Levij *Cancer Res.* **29**, 327; 1969; Polliack & Sasson *J. natn. Cancer Inst.* **48**, 407; 1972).

Retinoids and *in vitro* cell cultures

The problem with studying the action of retinoids *in vivo* or in organ culture is that there are too many pieces to the

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puzzle. One way round this is to look at the effect of retinoids, either alone or in combination with purified growth factors and tumour promoters, on cell lines in culture, when there is only one target cell type. For example, retinoids can be shown to restore anchorage-dependent growth to some transformed cells (Dion *et al. Expl Cell Res.* **117,** 15; 1978) and inhibit the growth of melanoma cells (Lotan *et al. J. natn. Cancer Inst.* **60,** 1035; 1978).

In a recent paper, Todaro *et al.* (*Nature* **276,** 272; 1978) reported that retinoids inhibit the mitogenic effect of sarcoma growth factor (SGF) on a line of untransformed rat fibroblasts. Earlier studies had shown that SGF production is switched on when cells are transformed by murine sarcoma virus. The polypeptide seems to promote cell division (either in the same cells or in other, normal lines) by binding at the plasma membrane to the site normally awaiting the arrival of epidermal growth factor (in spite of its name, EGF promotes cell division in a variety of 'fibroblast' cell lines as well as in epithelial cells) It has been argued that tumour promoters mimic SGF and EGF by short circuiting the membrane–intracellular effector signal system (Lee & Weinstein *Science* **202,** 313; 1978), and the results of Todaro *et al.* suggest that the retinoids may block the circuit altogether. Although still very speculative this model accounts for the opposite effects of retinoids and tumour promoters *in vivo.*

As usual though, things are not as simple as they seem and far from antagonising the effect of various promoters, it seems that retinoids can act like promoters in certain systems. For example, Wilson and Reich have recently described a culture system in which retinoids act synergistically with phorbol ester and Rous sarcoma virus in stimulating plasminogen activator in chick embryo fibroblasts (*Cell* **15,** 385; 1978). Plasminogen activator is an extracellular protease which is assayed *in vitro* by its ability to digest a fibrin clot, but very little is known about its role in fibroblast behaviour in the whole animal. It seems very likely, for example, that the enzyme is important in tissue remodelling during growth and wound healing. Until more is known about the effect of retinoids on these processes *in vivo,* Wilson and Reich argue for caution in interpreting all results according to a simple model.

Indeed, the same issue of *Cell* adds another facet to the problem of retinoid action in the form of a paper by Strickland and Mahdavi on the effect of retinoids on the differentiation of murine teratocarcinoma cells *in vitro* (*Cell* **15,** 393; 1978). These authors claim that retinoic acid promotes the differentiation of F9 embryonal carcinoma cells into parietal endoderm cells, which normally synthesise plasminogen activator and large amounts of basement membrane material. Similar results have been observed in M. Sherman's laboratory using a variety of synthetic analogues of retinoic acid (Jetten & Jetten *Nature,* in the press). Although the results suggest that retinoids can increase the (normally low) probability with which F9 cells switch to endoderm, the possibility that they are increasing the survival of endoderm cells formed 'spontaneously' in the cultures has not yet been completely eliminated

Meanwhile, readers who find these multifarious results too indigestible might ponder on a paper by Graham *et al.* (*J. natn. Cancer Inst.* **61,** 709; 1978) who report a lower risk of cancer of the colon and rectum associated with high intake of green vegetables. □

Free-electron lasers

from J. D. Lawson

SOURCES of incoherent radiation have been familiar since scientific enquiry began; the generation of coherent radiation, on the other hand, is a relatively recent achievement. Fifty years ago, coherent radiation with frequency up to about 10^7 Hz was available, and in common use for radio transmissions. More recently, however, a series of inventions, culminating in the laser, have pushed the frequency up by many orders of magnitude to the ultraviolet region of the spectrum.

Devices invented during the past 40 years include the klystron, cavity magnetron, travelling wave tube, ubitron, ammonia maser and, at higher frequencies, the optical laser and free-electron laser. All depend on the stimulated emission of photons. If the photons interacting with the charges are real, they constitute a radiation field, and the device may be termed a 'laser'. In some systems the electrons are essentially free, or move in orbits determined by external magnetic fields; in others they are bound to atoms in gases or in solids. Some devices require a quantum mechanical description, whereas others do not. The first analysis of the free electron free-electron laser was in quantum mehcanical terms, but classical theory is sufficiently accurate and is now always used.

The operation of two systems described as free-electron lasers has been reported, one at Stanford University (Deacon *et al., Phys. Rev. Lett.* **38,** 892; 1977) and the other at the Naval Research Laboratory, Washington (McDermott *et al., Phys. Rev. Lett.* **41,** 1368; 1978) These devices operate in different regimes, and both form an interesting link between conventional lasers and microwave tubes.

In most microwave generators the beam interacts with single mode cavities, or with structures carrying a slow wave with longitudinal phase velocity close to that of the electrons. Such cavities and structures are limited to transverse dimensions of less than about a wavelength, and this imposes a lower wavelength limit of a few millimetres for reliable devices with useful power output. This limit may be avoided by making use of interaction with a 'fast' electromagnetic wave, which has phase velocity equal to or exceeding that of light. Such interaction necessarily implies a velocity difference between wave and particles; although not synchronous, coherent interaction can nevertheless be obtained by modulating the orbit of the electrons This principle (Gorn *U.S. Patent 2, 591,* 350, filed 1947) has been used in the 'ubitron' millimetre wave generator, (Phillips, *ITED,* ED-7, 231; 1960), and the free electron laser represents a special application for relativistic electrons when the fast wave is essentially a plane wave travelling along the axis. I shall now describe the Stanford scheme.

Suppose that a circularly polarised light wave of wavelength λ travels in the z-direction. A particle travelling along the z-axis experiences a transverse force which rotates with angular velocity proportional to the difference between the electron velocity and the light velocity. If now a twisted transverse magnetic field of periodicity D is introduced on the axis (with field vectors corresponding to the edge of the stairs in a spiral staircase) the

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particle is constrained to move in a spiral orbit If this spiralling is synchronous with that of the electric field vector as 'seen' by the particle, then coherent interaction and continuous energy transfer is possible. For highly relativistic particles moving in a helix of radius $r \ll D$, simple geometrical arguments show that

$$\frac{\lambda}{D} \approx \frac{1}{2\gamma^2}\left(1 + \frac{4\pi^2\gamma^2r^2}{D^2}\right)$$

where γm_0c^2 is the electron energy and $\gamma \gg 1$. Thus, for reasonable values of D, (a few centimetres), and γ of order 50–100, coherent interactions may be obtained in the infrared or optical region. Freedom from the limitations of slow-wave systems has, however, been bought at the expense of a relatively weak interaction. This is because the electric field is almost perpendicular to the particle orbit.

A beam of electrons moving at approximately the synchronous velocity exchanges energy with the wave; if the average particle velocity is slightly greater than the synchronous velocity, energy transfer from the particles to the wave is possible. Two regimes may be distinguished, the low current regime in which the wave interacts essentially with individual particles, (as in an accelerator) and a high-current high-gain regime where the plasma oscillation frequency in the beam is no longer negligible, and the interaction is with a space-charge wave travelling on the beam. The Stanford and Columbia-NRL lasers referred to earlier operate respectively in these two regimes, which can be described in quantum language in terms of Compton and Raman scattering.

The first demonstration of the interaction mechanism outlined above was by Madey and his team at Stanford in 1975 (*Phys. Rev. Lett.* **36**, 717; 1976). Making use of the superconducting linac there, which is able to provide a continuous succession of short high quality pulses of electrons, amplification of 10.6 μm radiation from a CO_2 laser was demonstrated. The twisted magnetic field of 0.24 Tesla was provided by a superconducting bi-filar helix, of 5 m total length. This experiment was followed in 1977 by a further demonstration, using the same magnet and accelerator, of a laser oscillator. Mirrors 12.7 m apart were placed symmetrically about the helix, and the electrons were steered by magnets to avoid them at either end. A mean power of 0.36 W was measured, corresponding to a peak power of 7 kW during the pulse. The wavelength was 3.14 μm, and the linewidth of 0.23% was determined essentially by the very short pulselength of 4 picoseconds.

Such a laser has the very attractive feature that it can be tuned by varying the electron energy. Unfortunately, however, the apparatus required is expensive, and the overall efficiency is low. Furthermore, the linewidth is rather broad for spectroscopic applications The use of a storage ring to recirculate the beam and thus raise the efficiency has been suggested. Unfortunately, passage through the laser induces energy spread in the beam and this greatly reduces the efficiency on subsequent transits. Whether, and if so how, this effect can be overcome is still a subject of active debate.

It is too early to forsee what the applications of this type of laser will ultimately be. More study, both experimental and theoretical, is required before realistic assessments of power and efficiency limitations can be made; the gain decreases rapidly as γ increases and it remains to be seen just how the various technical constraints conspire to produce a lower wavelength limit. Experiments are difficult, and progress may not be rapid.

The laser recently described by groups at Columbia University and the Naval Research Laboratory, operates in the collective regime. A rippled solenoidal magnetic field is used, and interaction is with the slow, negative energy space-charge wave on the electron beam. (The system may be viewed in a frame of reference moving with the electron as a parametric amplifier, in which the rippled field and space-charge waves play the part of pump and idler.) The current in this laser is 25 kA, (about 10^4 times that in the Stanford laser), but the energy is much lower, corresponding to $\gamma=3.2$. 1 MW pulses of radiation of length 20 ns and wavelength 400 μm were extracted through the hole in one of the mirrors. This is not the first observation of microwave power from intense relativistic beams in a rippled field; it is however the first time that a laser cavity has been used to provide feedback and enhance the output. The spectral bandwidth was 2%; this arises from the difficulty of maintaining constant beam conditions during the pulse.

Whatever the final applications of the radiation from these devices, (a topic not discussed in the papers quoted), they form a promising addition to the available range of submillimetre generators. The latest volume of 'Physics of Quantum Electronics' (**5**, Addison-Wesley, Reading, Massachusetts, 1978) devoted entirely to this field includes an interesting variety of points of view of the theory, and a historical summary. ☐

Contractility of the roots of flagella and cilia

from Michael Sleigh

A HIGH proportion of the basal bodies of cilia and flagella bear striated roots and/or bundles of microtubular fibrils. In the absence of clear evidence for any other function it has been assumed that the purpose of these attached fibrous structures is to provide a firm anchorage for the ciliary base, so that the propulsive activity of the ciliary shaft can be exploited to the full. However, large striated roots are present not only on mechanically active cilia and flagella, but also on such sensory cilia as those in the rod cells of the vertebrate retina, where anchorage of the ciliary base would not seem to require such a large root structure. The recent report by Salisbury and Floyd (*Science* **202**, 975; 1978) that the striated roots of flagella of the algal flagellate *Platymonas* undergo a calcium-induced contraction should provoke further investigation of these structures.

The variation in the spacing of the major bands of striated roots has puzzled some electron microscopists for about 20 years; not only is there a wide range of periodicities in different species, varying between about 12 nm and about 200 nm, but there are also variations within species of between 30 and 60 nm in *Trichonympha* (Pitelka & Schooley *J. Morph.* **102**, 199; 1958) and between 13 and 25 nm in *Naegleria* (Simpson & Dingle *J. Cell Biol.* **51**, 323; 1971) Many authors noted a superficial similarity to collagen in the periodicity and appearance of these fibres, since in many cases periodicities of between 60 and 70 nm with several intraperiod bands were found, but the detailed banding pattern is different and a biochemical analysis of roots from mollusc cilia by Stephens (*J. Cell Biol.* **64**, 408; 1975), revealed the presence of a novel type of protein (ankyrin) that could be separated as a doublet with molecular weights of 230,000 and 250,000. Each striated root is composed of a bundle of longitudinal filaments about 5 nm thick, sharing the common banding pattern.

The two striated roots of *Platymonas* pass from the basal body complex at the base of an anterior depression of

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the cell across the centre of the cell close to the nucleus and terminate near the plasmalemma at the posterior end of the cell. The periodicity of these roots ranged from an extended spacing of 180 nm to a contracted spacing of about 60 nm. The longitudinal filaments were not visible in contracted roots, which were substantially (30%) fatter than extended roots. The extended pattern was seen in cells fixed after washing in artificial seawater lacking calcium or after washing in solutions containing EGTA, while the extreme contracted pattern was seen in cells treated with artificial seawater containing 2 mM $CaCl_2$; several other divalent cations were partially effective in causing contraction. Living cells observed in artificial seawater containing both 2 mM $CaCl_2$ and 5 mM ATP could be seen using the light microscope to undergo cyclic dimpling of the cell surface at sites of root attachment, and fixed cells from such experiments showed intermediate periodicities. This cyclical contractile activity of the striated roots is of particular interest, but the authors do not state its frequency, nor whether it coincides with any phase of the flagellar beat cycle. It is also of interest that the external application of ions and ATP is adequate to stimulate the contractile response in intact cells. The fully extended roots may be abnormal, since seawater contains calcium ions equivalent to about 10 mM $CaCl_2$.

Salisbury and Floyd suggest that the contraction of flagellar roots of *Platymonas* may have a function in the coordination or directional control of flagellar beating. This is interesting because in *Chlamydomonas* the two flagella are inserted at an angle, with the two basal bodies forming a V and a thick striated fibre (periodicity about 80 nm) connecting the upper parts of the two basal bodies (Ringo *J. Cell Biol* 33, 543; 1967). The response of *Chlamydomonas* to stimulation with calcium ions is a narrowing of the angle between the two basal bodies, perhaps by contraction of the thick striated fibre, and a transition from a 'breast-stroke' (cilium-like) beat that produces forward swimming to symmetrical undulations of the more parallel flagella that cause backward swimming (Hyams & Borisy *J. Cell Sci* 33, 235; 1978). It is also not impossible that the ciliary reorientation associated with the calcium-induced beat reversal of *Paramecium* could be associated with a contraction of the striated kinetodesmal rootlets (Naitoh *J. gen. Physiol.* **51**, 85; 1968).

Contractile activity of the striated roots of flagella and cilia of protists is in itself of interest, but if the striated roots of cilia in general are contractile, this finding could have wide implications, not least for sensory physiologists. For example, in the rod cells of the human eye a large striated root is attached to the basal body of the short cilium that connects the light-sensitive outer segment to the inner segment which leads to the sensory nerve; this root penetrates deep into the inner segment and ends in close contact with the plasmalemma of the inner segment. Some years ago Matsusaka (*J. Cell Biol.* 33, 203; 1967) showed histochemically that the ciliary roots in human rod cells possess ATPase activity. It may not therefore be too rash to speculate that the striated roots of the cilia in rod cells may be capable of contraction and that the cilium and its contractile root may be concerned in the transmission of information from the photoreceptive membranes of the outer segment to an electrically excitable membrane of the inner segment. ☐

Mediaeval meteors

from David W. Hughes

ASTRONOMICAL historians have been doing well during the past year, one of the high spots being the solution of a famous longstanding problem. No report had come to light in Europe and the Near East of any observations of the Crab supernova explosion which the Chinese apparently saw with great ease; it was first recorded on 4 July, 1054 and was so bright that it could be seen during the day-time for 23 days. K Brecher, E. Lieber and A. E. Lieber solved this puzzle (*Nature* **273**, 728; 1978) by finding a reference to the supernova in a biographical encyclopaedia of physicians written in about 1242 by Ibn Abi Usaybia. The compiler told how Ibn Butlan, a Christian physician, had seen the supernova when he was staying in Constantinople and had regarded it as being the cause of an epidemic which lead to the death of many thousands of people at that time.

A less newsworthy but more typical example of the work of the astronomical historian has been published recently in the *Journal for the History of Astronomy* (**9**, 123; 1979). Umberto Dall'Olmo has scoured the European annals and chronicles that have been collected in the *Monumenta Germaniae historica* and the *Rerum Italicarum scriptores* and has compiled a list of all the meteorites, meteors and meteor showers that occurred before 1500 AD and were regarded as being of sufficient importance to be recorded for posterity.

Dall'Olmo emphasises that his list is by no means complete. First it is obvious that many events went unrecorded. Second it is a hopeless task to examine all the historical and literary texts which mention astronomical events. The discovery of the Arabic record of the Crab supernova nearly a millenium after its occurrence is ample evidence of that.

Considering these facts it is fair to ask if lists of ancient astronomical events are of any use to the present astronomical community. The answer is an unequivocal yes. The period of accurate photographic and telescopic observations is so short in comparison with the age of civilisation, never mind the age of the Earth, that anything that extends this period is worthwhile. For this reason alone people have been searching for records of solar and lunar eclipses, comets, aurora, sunspots and meteoric phenomena. In the specific field covered by Dall'Olmo the occurrence, strength and date of meteor showers are vital parameters in a study of the perturbations that the causative meteor streams suffer during their lifetime in the Solar System. Meteor streams are moved by the gravitational attractions of the major planets and obviously can be detected from Earth only when the stream crosses the Earth's orbit.

These ancient records also provide a fascinating insight into the interests and priorities of the astronomers of the time. Meteors were regarded as ominous omens, but due to their brief duration they were only minor omens in comparison with solar eclipses and comets. A great shower in March 763 was interpreted as 'a forecast of the end of the world'. On 17 October, 855 meteors shot westward across the sky all night and 'for this reason Emperor Hlotarius prayed, neglecting all his affairs'. Man's great desire to link cause and effect is illustrated by the account of the fireball seen on 11 August, 1353 which left behind an ash-coloured vapour, twisted like a snake. The chronicler stresses that 'after its appearance it did not rain for three months' On 14 December, 1482, a very bright meteor was seen again in Parma, 'flying in a clear sky, and without noise —but there was an earthquake'...

It is also heartening to realise that meteorites were treasured in those days, just as they are now One that fell in Catalogne during 1348 was sent to the King on the back of a mule. A meteorite as large as the head of a man fell near Altesleibon in Germany in 1136, and 'the brothers of the abbey kept it carefully'. We read of a

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meteorite which fell in 586 'roaring with sparkles'. But my favourite account is of a rather unusual 'lapis mirae magnitudinis'. 'A stone as large as a house fell near Duringia, Germany in 1135, with a terrible noise that lasted for three days. The lower part of it, about half, sank into the ground, burning for three days like steel from the fire'. Also on July 26, 1243 'so many stars fell that if they had been true stars none would have remained in the sky'.

It is apparent that, in addition to their scientific worth, these historical papers are a source of some marvellous quotations. □

Man and desert

from Peter D. Moore

THE problems associated with the spread of deserts are both social and ecological. The involvement and interest of man in the process of desertification made it an appropriate subject for consideration at the Tenth International Congress of Anthropological and Ethnological Sciences*. It is also appropriate that it should have formed the theme of a post-plenary session entitled, 'Anthropology and Desertification' and have been held at the Central Arid Zone Research Institute (CAZRI), Jodhpur, for this institution has contributed much to the understanding of, and attack upon, desertification in the Rajasthan Desert of north-west India.

Complex historical and economic factors have contributed to human mismanagement of marginal arid lands. The indigenous peoples of the Rajasthan Desert, according to H. S. Mann and S. P. Malhotra (CAZRI), have many traditional techniques for coping with semi-arid conditions, including mixed farming, fragmented land holdings (of advantage in scattered rainfall areas), fallow and rotational farming systems, migration in famine, as well as socioreligious customs and taboos, particularly in relation to the care of trees. Modern desertification problems in the area may well be due to the imposition of new pressures rather than the long-term persistence of old ones.

A similar note, although with an economic overtone, was struck by B. Spooner (University of Pennsylvania). The modern economic system operates at a level greater than that of the ecosystem. Market forces are now the chief determinants in societies where once kinship and local organisation were most influential. Such a situation will encourage short-term exploitation of the local resources. Equally, the operation of rational, political processes at the new level may appear quite irrational at the ecosystem level. For this reason, even when ecological solutions to desertification problems can be envisaged, they may be difficult to implement locally. Historically, arid lands have often been politically independent and socially there may be resistance to innovative ideas from outside. It is vital, therefore, that the formulation of environmental policy should receive contributions from the indigenous society.

A novel and valuable feature of this conference was the involvement of anthropologists, environmental scientists and pastoral economists. The result was an unusual, yet productive, juxtapositon of disciplines and ideas. Among the pastoral economists, the contribution of S. Sandford (Overseas Development Institute, UK) is worth particular mention. He described pastoral systems as being either conservative or opportunistic. In the former, the number of grazing animals is kept at a constant, fairly low level. Since in most semi-arid areas there is considerable fluctuation in rainfall (and, therefore, in forage production) from year to year, the conservative system of pastoralism will keep overgrazing to a minimum, but will fail to exploit all of the available forage in a good year. An opportunistic system, on the other hand, varies the number of animals maintained according to the conditions prevailing. This should provide greater production efficiency, but in practice the number of stock is often reduced too little and too late, with consequent overgrazing and starvation. Environmentalists therefore tend to favour conservative strategies, but one is faced with the question of just how high the stock density should be set. In other words, how frequently can one tolerate the temporary over-exploitation of resources? If the answer is very infrequently, then stock density must be kept low, much of the resource is under-exploited and the entire system becomes far less attractive to the pastoralist than an efficiently managed, opportunistic system.

Specialised field studies and case histories in semi-arid areas are contributing much to understanding local problems. Several papers derived from a multi-disciplinary study of the Turan Biosphere Reserve of north-eastern Iran, a project directed by Spooner. C. Hamlin (University of Pennsylvania) described the use of satellite remote sensing for the detection of changes in vegetation and sand distribution. A. E. Nyerges has examined the feeding behaviour of sheep and goats, which are herded in mixed flocks in the Turan. Adult goats proceeded at a rate of 25 m min^{-1} whilst feeding and this allowed them to range further from villages when foraging than sheep, which proceeded at 17 m min^{-1}. Kids achieved only 14 m min^{-1}. The result of these different speeds is that a zonation of grazing pressures exists around villages and pens, in which distant areas are predominantly grazed by goats. The selectivity of the two animals also varies, for example, *Artemisia* comprises 23% of the sheep's diet, but only 15% of the goat's intake. Thus, both qualitative and quantitative variations in vegetation predation exist at different distances from a village.

A further pressure on vegetation associated with human settlement is the demand for fuel wood. M. Martin has estimated that a family requires 150 kg wood each month. The processing of milk products from a flock of goats

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*Held in Delhi on 12–18 December, 1978. It was convened by B. Spooner (University of Pennsylvania) and H. S. Mann (Director of CAZRI).

A hundred years ago

IN connection with our article last week on a proposed Scottish observatory, it may be interesting to state that one day last August Mr. Milne Home, chairman of the Meteorological Society for Scotland, accompanied by Mr. Colin Livingston, headmaster, Public School, Fort William; Mr. Thompson, student; and Mr. David Doig, contractor, ascended Ben Nevis and made several observations with the view of erecting a station on the summit. They found the top enveloped in a mantle of snow—a circumstance which rendered it an extremely difficult task to select suitable spots for the erection of a dwelling-house and observatory. After a careful survey Mr. Home came to the conclusion that the plateau immediately beyond the spring affords the best site. The recommendation this spot has is its contiguity to the water-supply. It is proposed to construct the buildings after the following plan: first, a wall of stone with an inside lining of wood and an inner coating of felting, and the outside of the wall to be covered with corrugated iron. An external wall of stone would also be erected to serve as a protection from the blast. The estimated cost of the structure is 500*l*.

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and sheep may require as much as 100–150 kg each day. Thus the total pressure is considerable in a habitat where, according to R. Bhadresa (King's College, London) the woody biomass above ground is only about 2,600 kg ha^{-1}.

One conclusion which emerges from an interdisciplinary study of this sort is that the ecological questions, such as those concerning the extent of human impact, are relatively easy to answer compared with the social problems which are likely to arise if attempts are made to reduce this impact. ☐

Galaxy clustering and anti-clustering

from William C. Saslaw

THE positions of galaxies on the sky contain much important astrophysical information. First, their spatial distribution provides a link with the conditions of their formation and the large scale expansion of the Universe. Second, their distribution may show evidence for haloes of absorbing gas or dust around galaxies Interesting developments are occurring in both these areas.

In the standard view of our expanding Universe, galaxies formed at a redshift $Z \lesssim 10^3$, after matter and radiation decoupled. We do not understand the detailed manner of their formation, nor do we know their initial distribution in space. Having formed, however, galaxies interact gravitationally as massive point particles and we can follow their changing distribution from some assumed initial state. The encouraging result is that the simplest initial state—a uniform random distribution—can lead to the main properties of the non-uniform clustering we now see.

This clustering of galaxies was first observed by Herschel and analysed by von Humboldt (*Cosmos*, 1866) even before they knew the nature of the nebulae. More recently, one of the most useful measures of clustering has turned out to be the two-point galaxy correlation function, $\xi (r)$, discovered by Totsuji and Kihara (*Pub. Ast. Soc. Japan* **21**, 221; 1969) who analysed the Lick survey of galaxies brighter than 19th magnitude. They found that the difference between the observed probability of finding a galaxy at distance r from any arbitrary galaxy and this probability in a uniform random distribution, varies approximately as $r^{-1.8}$ over distances between about 0.5 and 10 megaparsecs. Their results have since been confirmed and extended by many astronomers, especially Peebles and his collaborators at Princeton.

To help understand these observations, several theoreticians working mainly with N-body computer codes developed by Aarseth at Cambridge have simulated the clustering of point masses in expanding universes. One of the major questions is to discover what ranges of initial clustering spectra and expansion rates are compatible with the observed two-point and higher order correlations. A forthcoming paper by Efstathiou (*Mon. Not. R. Astr. Soc.* **186**, 133; 1979) helps to clarify this question. He computed two 1,000-body clustering problems. Both started from exactly the same Poisson initial distribution. One evolved in a closed Einstein–de Sitter universe (which has density parameter $\Omega = 1$). The other evolved in a more rapidly expanding open universe which started with $\Omega = 0.775$ and ended with $\Omega = 0.1$ after the expansion radius had increased by a factor of 31. The galaxies in the closed universe evolved into a distribution $\xi (r) \sim {}^{-1.8}$ after the universe expanded to several times its original radius. This agrees with several previous simulations by others. The galaxies in the open universe, however, had a somewhat steeper correlation function at each stage of its expansion. Thus at any given chronological time, the expansion rate seems to be mirrored in the galaxy correlations.

Taken at face value, this would support the view that we live in a closed universe. However, as Efstathiou recognises, this would be a premature conclusion. One reason is that fluctuations of ~ 0.2 in the exponent of $\xi (r)$ are found among different 1,000-body simulations using the same initial statistical (but not identical) distribution To reduce these 'ensemble fluctuations' one needs to use more particles. At present it is possible to compute $\sim 4{,}000$-body problems in reasonable time using direct integration methods There are plans to evolve about 20,000 bodies using Fourier techniques under conditions suitable for finding two-particle correlation functions, and these should produce results soon. Another difficulty is to sort out the results of different initial distributions of galaxies. Here one especially wants to learn whether the initial distributions which would evolve into the observed distribution using a rapidly expanding open universe need to be very *ad hoc*. In the more distant future, enough galaxy redshifts could be measured to make use of constraints from velocity correlations as well as spatial correlations.

We turn now to a second aspect of the galaxy distribution: anti-clustering. Unlike the positive correlations just discussed which are found using a huge sample of galaxies, anti-clustering may be found among small subsets. These subsets make a negligible contribution to the overall correlation function, but are of special interest on their own. In particular, if there is a relative deficit of distant galaxies around nearby ones, the most plausible explanation would be an obscuring halo of gas or dust around each of the nearby galaxies. Such haloes, with a mass at least several times the mass in the easily visible body of the galaxy, have become fashionable in recent years as a means of stabilising spiral galaxies against the formation of a bar in their centre. It is now doubtful whether such a massive halo is really necessary for stability. This stability can also be provided by counterstreaming stars (Kalnajs, *Astrophys. J.* **212**, 637; 1977; Zang & Hohl *Astrophys. J.* **226**, 521; 1978) which remain from mergers which formed the galaxy, or by a concentration of mass in the nucleus of a galaxy. Moreover, it is not clear that the nonlinear results of instability to bar formation, possibly followed by a transition to spiral or elliptical structure in an explosion or gravitational encounter, are inconsistent with observations Nevertheless, the existence of galactic haloes remains an important question. For example, if most galaxies have haloes of about 10 times their presently observed mass, it would strengthen the evidence that we live in a closed universe.

Direct evidence for haloes could come from faint optical or X-ray emission around galaxies, from the rotational velocity of neutral hydrogen clouds around galaxies, and from gas or dust around nearby galaxies which absorbs the light of more distant galaxies. Observations with the first two techniques suggest that haloes do exist around some galaxies, but their masses are very uncertain. In the case of optical or X-ray emission we do not know the mass/luminosity ratio. This ratio depends on both the composition and geometric distribution of material in the halo. With neutral hydrogen clouds, the masses can be determined more accurately but the observations are not easy to make. They suggest haloes of moderate mass.

The third technique, galaxy anticlustering, has not been pursued vigorously This is because, until very recently, there was not a large enough

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complete sample of galaxies with known distances in which to look for statistical anti-clustering between near and distant galaxies. The relative distances had to be estimated from rather fickle secondary indicators such as apparent magnitude or size. Using these secondary indicators, Bogart and Wagoner (*Astrophys. J.* **181,** 609; 1973) found weak evidence for anti-clustering between near and distant rich clusters of galaxies, and Wesson (*Astrophys. Lett.* **19,** 127; 1978) has claimed to find this effect among 274 bright galaxies in the local supercluster. Now, however, many more galaxy redshifts are becoming available and they will enable much better analyses of anti-clustering based on distance to be made. Sandage (*Astrophys J.* **83,** 904; 1978) has published 719 redshifts for galaxies in the local supercluster, and more are to come. Recently Vera Rubin of the Carnegie Institute in Washington DC has compiled a list of lists of redshifts published in 1976–1978 and of measurements in progress. More than 5,500 redshifts were published in this period and a roughly equal number should be measured in the next year or two. This will be a sample to conjure with. In addition, powerful new machines, such as the one which Kibblewhite and his associates are completing in Cambridge, will determine the positions and characteristics of galaxies on photographic plates rapidly and automatically. Thus in the years just ahead we can expect to understand much more about the distribution of matter over the largest scales of the Universe.

Gravitational waves confirmed at Texas Symposium

from M. C. Begelman, A. C. Fabian, N. A. Sharp and A. Wakelin

THE peripatetic Texas Symposium on Relativistic Astrophysics went to Munich in 1978* with, for the first time, participants from the People's Republic of China. Traditionally, the symposium is not regarded primarily as a forum for announcing the latest results, but more as a chance to review the progress of the past 2 years. With several exceptions—notably the new findings on the binary pulsar and the discovery of a new type of emission-line object—this meeting largely followed suit.

The first day was devoted to extragalactic sources, which, to the relativistic astrophysicists, meant quasars, BL Lacertae objects, and Seyferts. There was little theoretical debate, black holes and beams are seemingly well entrenched as the current fashion, and most of the reported progress was observational. It seems that the defining characteristics of the various types of object are still controversial. Several speakers (and recent observations) suggested that common classification schemes, based on spectral signature, may be misleading. For instance, it seems that all Seyferts may radiate the bulk of their energy in X rays, and, so far, there are three known X-ray quasars. Infrared also figured strongly, but sadly there was no discussion of the wealth of ultraviolet data now available from the IUE satellite.

Of particular cosmological interest was the report on the microwave background, devoted mainly to the very careful work of D. P. Woody and P. L. Richards of the University of California Lawrence Berkeley Laboratory. The spectrum now appears to be only approximately black body with a temperature of ~3K, being instead too peaked and too narrow. This deviation cannot be explained by any of the processes currently expected to produce deviations. Further theoretical work and further observations will doubtless be available by the next Texas meeting.

There was also a very lucid review by G. F. R. Ellis (University of Capetown) of the many assumptions and corrections which are made by cosmologists when considering the available data. Although much of this is known to the expert practitioner it deserves a wider audience amongst all people collecting and interpreting data of cosmological interest.

It was not until halfway through the conference that classical relativity theory made its appearance, with a clear discussion by J. Ehlers (Munich) of the difficulties of treating isolated systems. This is of great importance in the interpretation of the binary pulsar.

The common meeting ground for relativists and astronomers is this binary pulsar. Discovered in 1974 by Hulse and Taylor, the pulsar swoops around its companion in a highly elliptical orbit at velocities ~0.1% of the velocity of light. J. Taylor (University of Massachusetts at Amherst) reported the detection of four more relativistic effects, the most exciting of which is the confirmation of the existence of gravitational waves. He announced the measurement of the advance of periastron (similar to the excess advance of the perihelion of Mercury attributed to relativistic effects) at an earlier Texas conference. Refinements in the measuring equipment and techniques have allowed Taylor, and his colleagues L. Fowler and P. McCulloch, to improve the timing accuracy of the pulse arrivals by a factor of 20. An iterative method has then been used to obtain corrections up to the third order in (v/c).

In particular, the rate of decrease of the orbital period is observed to be $\sim -3.2\times10^{-12}$ s s^{-1}, and is of the correct sense and magnitude to agree with the general relativity prediction of energy and angular momentum loss by way of gravitational radiation. The importance of gravitational radiation in causing the spiralling together of binary companions has long been realised in the evolution of close non-pulsar binary system. This is the first time that a quantitative measurement has been made. The binary pulsar is apparently turning out to be a remarkably clean laboratory for relativistic astrophysics. Further work, especially theoretical, will, it is hoped, check all other possible causes of the observed effects and refine the measurements already made. The current data restrict the masses of the pulsar and its companion to around 1.4 $M_\odot$ each, consistent with a pair of neutron stars, or a neutron star (the pulsar) and a white dwarf. Changes in the observed pulse shape hint at spin-orbit coupling causing the pulsar spin axis to process.

J. Nelson (Lawrence Berkeley Laboratory) reported on an optical object found by Crane within 0.3 arcsecs of the position of the binary pulsar. If it is the pulsar, then the pulsed fraction is less than 1%, and if it is the companion then it must be hotter than ~18,000 K in order to be able to radiate the observed flux without causing an excessive periastron advance.

The discovery of the most unusual stellar spectrum of 1978 was undoubtedly reported by B. Margon (University of California, Los Angeles). The 14th magnitude emission line object numbered 433 in the catalogue of Stephenson and Sanduleak shows emission lines that move across the spectrum! These strong features have been seen to shift by up to 600 Å (an equivalent velocity shift of ~0.1 c) in 30 days. SS433 has been the subject of intense study recently since it was

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*The 9th Texas Symposium on Relativistic Astrophysics was held in Munich on 14–19 December, 1978.

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realised that it was associated with a variable radio source (see for example Clark & Murdin, *Nature* **276**, 44; 1978; Ryle *et al. Nature* **276**, 571; 1978). An X-ray source A1909+04 is also in the right position and it is located in line with the supernova remnant W50. Margon and his colleagues are the first to observe the unusual optical emission features. Two in particular lie either side of Hα, the infrared one of which moved to longer wavelengths by 150 Å, the red feature moving in the opposite sense by 70 Å, in just four nights observing. SS433 presents a challenge; perhaps it too will become a regular at the Texas conferences. □

Pathogenic mechanisms of mycoplasmas

from D. B. Archer

MYCOPLASMAS are a diverse group of wall-less prokaryotes. They have been implicated as possible causal agents in a multitude of diseases in animals, plants and insects, but have been demonstrated to be causative in relatively few instances and in only a handful of cases is there any information which bears directly on the pathogenic mechanisms involved. However, from the Proceedings of the 2nd Conference of the International Organisation for Mycoplasmology (IOM) (*Zentralbl. Bakt. Parasitnek. Infekt. Hyg.* **241**; 1978) it is clear that an understanding of the pathogenic mechanisms is now being worked towards.

The diversity in form of mycoplasmas reflects the range of diseases and mechanisms involved. Some mycoplasmas are motile and motility may be necessary for these organisms to reach their target site, although a non-motile variant of *Spiroplasma citri* is known to be pathogenic when injected into host phloem cells by an insect vector (Townsend *et al. J. gen. Microbiol.* **100**, 15; 1977). Mycoplasmas lack flagella and their mechanisms of motility are poorly understood although an actin-like protein has been reported in *Mycoplasma pneumoniae* (Neimark *Proc. natn. Acad. Sci. U.S.A.* **74**, 4041; 1977). However, Rodwell *et al.* (*IOM Proceedings*) showed that no mycoplasma protein co-electrophoresed with rabbit muscle actin by two-dimensional gel electrophoresis. Although Rodwell *et al.* could not detect a mycoplasma protein having a high affinity for deoxyribonuclease 1, a characteristic of eukaryotic actin, Kahane *et al.* (*IOM Proceedings*) were able to isolate a multiprotein complex having a high affinity for muscle myosin.

Attachment to host cells is of primary importance in the pathogenicity of *Mycoplasma gallisepticum* and *M.pneumoniae*, among others. A *M.pneumoniae* membrane protein (P1) is required for the attachment to host tracheal cells (Hu *et al. J. exp. Med.* **145**, 1328; 1977) and although these workers could not detect any staining of *M.pneumoniae* proteins with PAS, a carbohydrate stain, it is of interest to know whether P1 is identical to the recently isolated glycoprotein from *M.pneumoniae* (Kahane & Brunner *Infect. Immun.* **18**, 273; 1977) because carbohydrate has been implicated in the attachment of this organism to host epithelium (Powell *et al. Infect. Immun.* **13**, 959; 1976). Apart from the presence of a glycoprotein in *M.pneumoniae*, and possibly *M.gallisepticum* (Goel & Lemcke *Ann. Microbiol.* **126**, 299; 1975) there are no confirmed reports of glycoproteins in any other mycoplasmas. If carbohydrate is important in the host cell attachment of other mycoplasmas it need not be through a glycoprotein and Huang (*Nature* **276**, 624; 1978) has recently pointed out that glycolipids can be involved in cell adhesion, although glycolipids do not seem to be involved in the host cell adhesion of *M. pneumoniae* (Hu *et al. op. cit*). Both *M. pneumoniae* and *M. gallisepticum* adhere to erythrocytes and this attachment has been used as a convenient model for natural host cell attachment. It seems that glycophorin is the major erythrocyte receptor for *M. gallisepticum* (Banai *et al. IOM Proceedings; Infect. Immun.* **21**, 365; 1978) whereas this is not the case with *M. pneumoniae* (Feldner *et al. IOM Proceedings*), although bindings of *M. pneumoniae* to host epithelial cells is reduced by pretreating host cells with neuraminidase (Powell *et al. Infect. Immun.* **13**, 959; 1976; Gabridge, *IOM Proceedings*). These mycoplasmas also bind to glass and plastic so it is of interest to know what determines specificity of binding *in vivo*. Sialic acid is of importance in the binding of *M.gallisepticum* to erythrocytes but this residue alone probably could not provide specificity of host. Isolation of the presumed binding proteins of mycoplasmas and determination of their affinities for target sites would enable some judgements to be made on the specificity of mycoplasma–host cell interactions.

Host cell attachment may not be as important in determining specificity as the production of a mycoplasma toxic substance or susceptibility of the host to it. Indeed, there is no evidence for high affinity host cell attachment by most mycoplasmas. Unfortunately, information on toxic products of mycoplasmas is meagre. Hydrogen peroxide and ammonia are produced by some mycoplasmas and may be toxic factors, particularly if the association of the mycoplasma with the host is intimate (for example *M. pneumoniae*) or if the amount of hydrogen peroxide is large (*Mycoplasma mycoides* subsp. *capri*). Specificity may more easily be explained by the production of a toxic compound which has selectivity of action, but little information on such substances is available. Characterised toxic cell products are the galactan of *M.mycoides* subsp. *mycoides* (Lloyd *et al. J. med. Microbiol.* **4**, 425; 1971: Buttery *et al. J. med. Microbiol.* **9**, 379; 1976) and the exotoxin of *Mycoplasma neurolyticum* (Tully, *INSERM* **33**, 317; 1974). Also, low molecular weight toxins have been isolated from *S.citri* (Daniels & Meddins *INSERM* **33**, 195; 1974: Daniels *IOM Proceedings*). One hopes that purification and characterisation of such toxic products will lead to experiments designed to determine their modes of action. Exotoxins were not detected, however, in culture filtrates of *M. gallisepticum*, an organism known to be neurotoxic (Thomas *Ann. N.Y. Acad Sci.* **143**, 218; 1967). Some mycoplasmas bind to and transform lymphocytes (Ginsburg & Nicolet *Nature new Biol.* **246**, 143; 1973; Cole *et al. IOM Proceedings*; Wise *et al. IOM Proceedings*) and form caps (Stanbridge *IOM Proceedings*; Stanbridge & Weiss *Nature* **276**, 583; 1978) demonstrating mitogenic activity *in vitro*. Also, Cole *et al.* (*IOM Proceedings*) reported that mycoplasmas induce the release of cytotoxic factors from lymphocytes. The *in vivo* importance of mycoplasma interactions with lymphocytes is unknown but provides a plausible explanation for many of the effects of mycoplasma infection.

In conclusion, although information on the pathogenic mechanisms of mycoplasmas is still limited the situation is improving. Specificity remains a major problem which can only be overcome by characterisation of the cell residues involved in host cell attachment of those mycoplasmas which do attach, and by isolation and characterisation of toxic factors. Demonstration of *in vivo* mitogenicity would clearly be a major advance in our understanding of the pathogenic mechanisms of mycoplasmas. □

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articles

Lowland Maya radiocarbon dates and the classic Maya collapse

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Three processes of the Late Classic Maya collapse, the demise of the stela cult, the breakdown of elite culture, and the reduction of the commoner population, are compared by frequency-distribution bar-graphs of dated monuments in the Southern Lowlands and radiocarbon dates from elite and commoner contexts. The demise of the stela cult and the breakdown of elite culture seem to have coincided. The commoner radiocarbon bar-graph does not necessarily indicate the occurrence of a major demographic catastrophe.

THE Late Classic Maya collapse poses one of the most challenging problems in world archaeology. Briefly, it represents an abrupt cessation of Lowland Maya elite class culture—particularly such activities as the use and production of carved and dated monuments, platform-temples, elaborate residential structures, fine polychrome pottery, and carved jade—during the ninth and early tenth centuries AD. The demise of the elite class was apparently accompanied by a rapid and large-scale depopulation of the commoner class, particularly in the Southern Lowlands (eastern Chiapas, the Peten and western Belize).

Our study is intended not to explain the events of the collapse, but to empirically measure the rate of decline in several cultural processes integral to the collapse. Three phenomena of particular interest are the cessation of the 'stela cult'; the decline in the general activities of the elite class; and the apparent population reduction of the commoner or peasant farmer class. For each of these cases we have produced a 'decline curve' that is based on a frequency-distribution bar-graph of relevant chronometric data, namely the dedicatory Long Count dates inscribed on monuments, and radiocarbon dates from elite or commoner contexts.

Traditional theories have tried to explain the collapse from either an internal or external orientation[1,2]. The internal theories emphasise either natural catastrophes such as overpopulation, soil exhaustion, disease, earthquakes, hurricanes, climatic change, and insect pests; or socio-political crises such as peasant revolts and intersite warfare. The external theories view the collapse in terms of cultural developments, such as military or economic events, throughout the whole of Mesoamerica. Unfortunately, the majority of these hypotheses are speculative single-cause theories which are not supported by statistically valid empirical data.

A new research phase on the Maya collapse was initiated in 1970 at a seminar by the School of American Research, Santa Fe, New Mexico[3]. The theoretical model, summarised by Willey and Shimkin[4], is tailored to future hypothesis-testing. This model states that certain activities of the elite class which had successfully stimulated the growth of Early Classic Maya civilisation became inappropriate for a new Late Classic society faced with a burgeoning population, precarious agricultural production, and military harassment by central Mexican groups. The cumulative effect of these disrupting factors was to administer a fatal shock to the Maya political system. The demise of the elite class is thought to have been marked by the erection of the last calendrically dated stelae at Seibal and Uaxactun in AD 889 or in Tonina in AD 909; the termination of monumental architecture at Tikal in AD 830, Altar in AD 909 and Seibal in AD 928; and the appearance of the Tepeu 3 ceramic horizon in the ninth century AD that signalled the end of the Peten polychrome tradition and the appearance of foreign fine paste wares. Following the tenth century no general recovery of Classic Maya civilisation took place in the Southern Lowlands. Some pockets of elite tradition continued to exist, however, along with reduced commoner populations, at such sites as Tikal, Altar and Seibal[4].

Analysis of stelae dates

The Sante Fe model relies heavily on stelae dates to construct a chronology for the collapse of the Maya elite. Yet it fails to provide a comprehensive frequency-distribution of Lowland stelae erection. This is done in the following section, as a test of the Santa Fe model.

The erection of stelae and altars was an important activity for the Classic Maya. The labour necessary for the erection of these multi-ton limestone monuments ensured that the stela cult crosscut Maya religion, politics and economics. The quarrying and sculpting, for example, was probably done by professional masons and artisans. Many dozens, and sometimes several hundreds, of labourers had to drag the megalith to the site. The average Peten stela had a mass of about 5 metric tonnes, while the largest in the Lowlands (stela E at Quirigua) had a mass close to 50 tonnes[5]. The elite no doubt maintained a high visibility as the organisers of this impressive operation—because precisely such displays of power helped to maintain their authority. The upright rectangular stela, featuring a life-size low-relief portrait of the ruler, often colourfully painted, had a hieroglyphic text with such information as the ruler's genealogy and the dates of his accession to the throne, marriages, battles, and so on. Smaller cylindrical altars, usually with a shorter text and less sculptural ornamentation, were placed in front of the stela. In addition to their role as a power displays for the elite, the stelae and altars also had important religious functions. They were usually erected on 'period-endings' that commemorated important calendric periods such as Katuns and Hotuns (9.9 yr). Their hieroglyphic texts contain substantial technical information on calendars and other ritualistic activities. Finally, ceremonial caches were frequently buried underneath or alongside the stela. These monuments clearly served as foci for both political and religious activities.

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The number of dated Maya monuments, both stelae and altars, erected in the Southern Lowlands per Katun cycle (19.8 yr) during the period AD 292–909 is shown in Fig. 2. The span of time ranges from the erection of the earliest long count-dated stela yet found in the Lowlands (Tikal St 29) at AD 292, up to the last securely-dated Lowland stela at Tonina. Graphs similar to Fig. 2 have been previously published[6–8] but to our knowledge Fig. 2 is the most comprehensive for the Southern Lowlands. It includes 415 Long Count dates, most of them period-endings, marked on 359 stelae and 56 altars from 62 sites. The boundary for the Southern Lowlands is marked in Fig. 1. Southern Lowland sites from which we obtained either stelae or altar dates for our study are marked in Fig. 1 by a triangle, while sites that furnished radiocarbon dates are marked by a star. The area that we have marked as the Southern Lowlands is almost identical to that used by the Sante Fe Seminar[3]. (The stelae data[5] were obtained from the literature and also from personal communication with J. Graham and I. Graham.)

Recent evidence suggests the possibility that Protoclassic rulers (AD 100–250) of Highland Guatemalan centres, such as Kaminaljuyu, exported the stela cult to the Lowlands by military conquest or marriage alliance[9]. Figure 2 shows that few dated monuments were erected in the Lowlands before AD 475 (9.2.0.0.0.). Sites with these early monuments are clustered within a 30 km radius in the north-east Peten. Following the major waterways, however, the stela cult moved rapidly into neighbouring regions and by AD 534 (9.5.0.0.0.) the maximum geographical spread for Classic Maya stelae had been reached[10]. Shortly afterwards the growth of the cult was slowed by a brief and enigmatic hiatus that lasted from AD 534–613 (9.5.0.0.0.–9.9.0.0.0.) which apparently was caused by the withdrawal of Central Mexican political influence in the Peten[11].

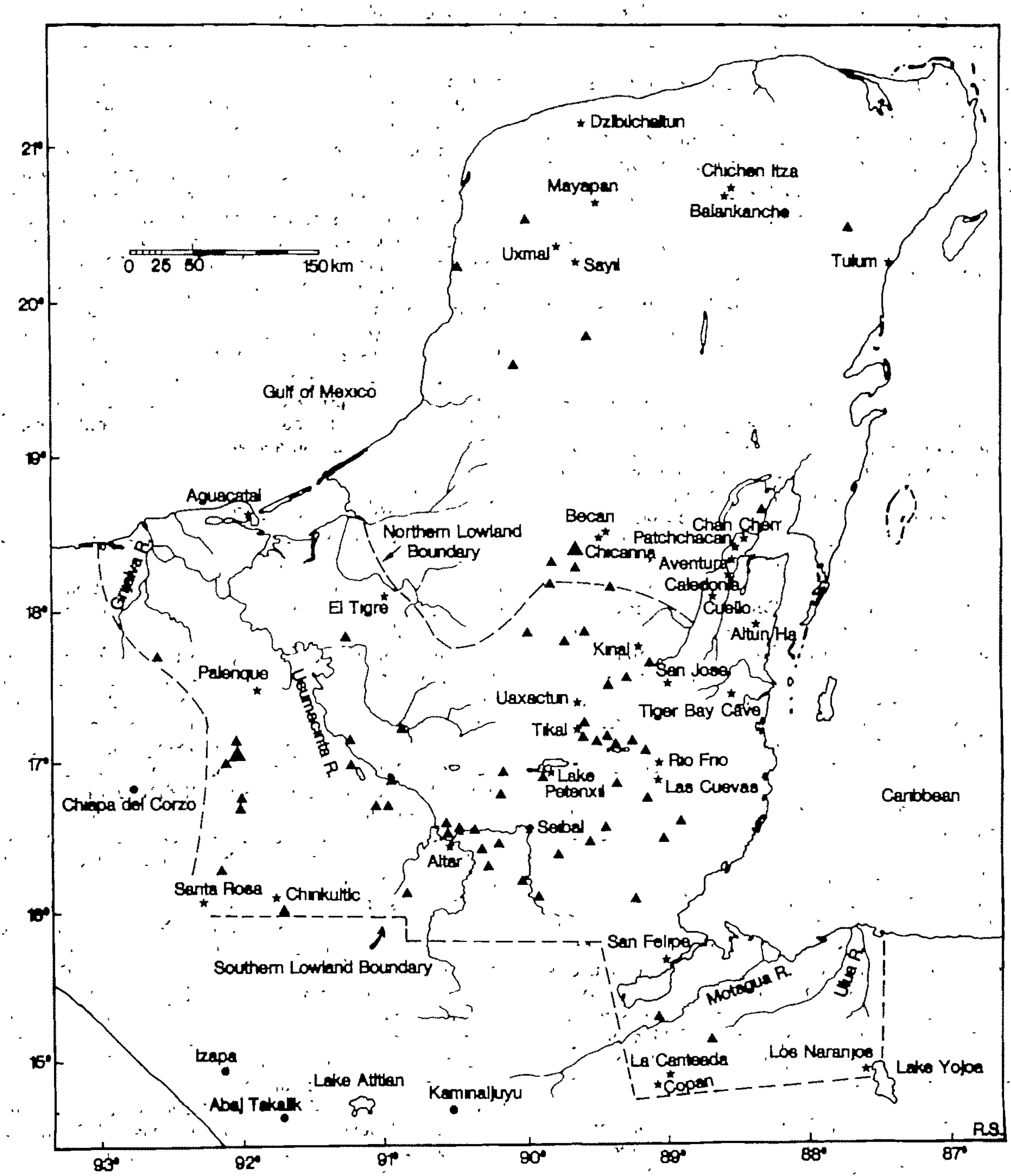

Fig. 1 Map of Maya Lowlands showing sites with dated monuments and radiocarbon dates. Only Southern Lowland sites were used in study. Note both long count dates and radiocarbon dates were used from Tikal, Uaxactun, Palenque, Seibal, Altar and Copan. The last Terminal Classic stelae were erected at Tonina, Chiapas and San Lorenzo, Campeche (sites marked by large triangles).

The explosive growth of the cult during the period AD 613–790 (9.9.0.0.0.–9.18.0.0.0.) is clear in Fig. 2. During this period at least 324 stelae and altars were dedicated in both the Southern and Northern Lowlands. The climax of the stela cult was reached in the eighth century. This zenith can be measured by several statistical conventions. The mean stela date, calculated from a sample of 300 stelae, is AD 705 (ref. 5). The date AD 731 (9.15.0.0.0.) is the statistical mode, as it occurs on more monuments than any other period-ending[12], while the date AD 790 was commemorated by at least 19 ceremonial centres, more so than any other period-ending[6,7].

The popularity of the cult took a sudden downwards plunge at AD 810 (9.19.0.0.0.). Its rapid decline culminated with the erection of the last securely-dated stela at Tonina, Chiapas in AD 909 (10.4.0.0.0.) (I. Graham, personal communication). We include in Fig. 2 an even later stela at San Lorenzo, Campeche which has been dated by J. E. S. Thompson to AD 928 (10.5.0.0.0.)[13]. Its absolute date cannot be established conclusively, however, as it bears only a floating Calendar Round date.

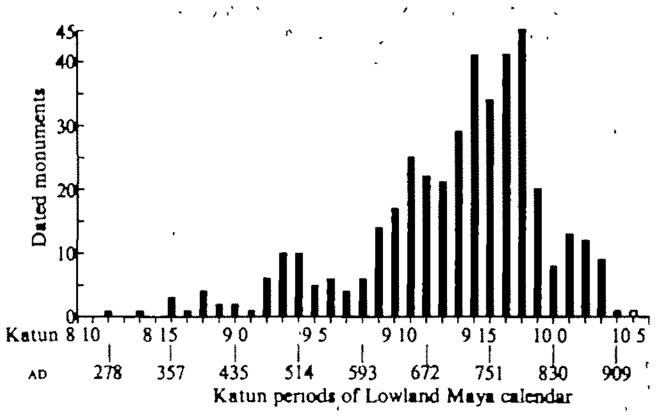

Fig. 2 The number of dated monuments erected per Katun period in the Southern Maya Lowlands. All monuments that were erected during a given Katun period are indicated in the position directly above the start of the Katun. Included are: 415 LC dates carved on 359 stelae and 56 altars from 62 Southern Lowland sites.

Figure 2 shows that a genuine collapse or abrupt breakdown of the stela cult occurred, rather than a gradual or normal decline in popularity, by both the asymmetry of the distribution and the steep downslope. The growth and maturity of the cult lasted some 500 yr, whereas its decline took place in about a century. This sudden loss of an established tradition of ruler glorification does favour the demise of the rulers themselves and their entourage.

Bar-graphs of radiocarbon dates

In their model Willey and Shimkin[4] found that the degree of commoner population decline following the loss of the elite was the most formidable research problem of the Maya collapse. They did not specify the exact degree to which populations of the Southern Maya Lowlands either died out or migrated. Adams, on the other hand, apparently relying on ceramic evidence, has estimated that an unusually rapid depopulation from 3,000,000 to 450,000 took place in the countryside and at ceremonial centres in a period from 75 to 100 yr (ref. 14). Similarly, Culbert offers a population loss for the Lowlands in excess of a million people within a century and describes it as one of the world's greatest demographic disasters.

In his synthesis of ceramic chronologies for the Santa Fe Seminar, Rands demonstrated that in general the average Maya ceramic period lasted 75 yr, with an uncertainty of ±30 yr for either the beginning or ending date[8]. Thus ceramically-based population studies at any given Maya site can only suggest that a house mound had been occupied during part or all of a 75 yr period. A further complication is the problem of the terminal stratigraphic layer, which in many cases represents the transitional Late Classic–early Postclassic occupation of the site. This stratum is particularly troublesome because it lacks a bordering stratum that can fix an incontrovertible upper temporal limit[3].

Frequency-distribution bar-graphs of Lowland Maya radiocarbon dates offer an alternate or supplementary dating method necessary in establishing rates of decline for non-stelae dated Late Classic elite activities and the relative population size of the commoner class. A large number of Maya radiocarbon dates already exist, and many others could be readily obtained from the hundreds of skeletons and charcoal samples that have been collected and stored during major Maya field projects by Canadian, American, Mexican and Guatemalan academic institutions. The average range of uncertainty associated with Maya radiocarbon dates so far collected is of the order of ±80 yr, which is similar to the time span of the average Maya ceramic phase.

To determine whether elite activities in general did undergo a radical decline at the same time as the collapse of the stela cult, we have produced a frequency-distribution bar-graph of 96 radiocarbon dates whose archaeological context represents various Southern Lowland Maya elite activities. Figure 3*a* shows the frequency of these activities through the period 51 BC to AD 1389. These activities, as dated by the ^{14}C method, include the inhabitation of palaces, the building of platform-temples and palaces; the production of sophisticated carved wooden lintels for palaces and temples; the production of elegant wooden figurines and bark-paper codices; and the administration of cave ceremonies and elaborate burials.

Most of the 153 radiocarbon dates in Table 1 have already been published and were compiled through an intensive search of the literature. Table 1 also includes 22 previously unpublished ^{14}C dates from Tikal, Guatemala, that were measured by the University of Pennsylvania Radiocarbon Laboratory (W. Coe and E. Ralph, personal communication). Table 1 is intended to be a comprehensive list of most of the ^{14}C dates presently available from the Maya Lowlands during the period 51 BC to AD 1389. Accordingly, it contains an additional 27 dates from the Maya Northern Lowlands that are not plotted in Fig. 3*a*, which are marked by an asterisk. Table 1 gives the archaeological provenience of the ^{14}C sample and classifies this provenience into an elite or commoner context. The classification was made by us on the basis of the excavator's description of the excavation context. All radiocarbon dates, whether previously published or not, were converted to tree-ring calibrated dates using the chart of Suess[16,17]. For purposes of standardisation the calibrated date was rounded to the nearest interval of 50. Accordingly our calibrated date may differ slightly from previously published versions. Any other

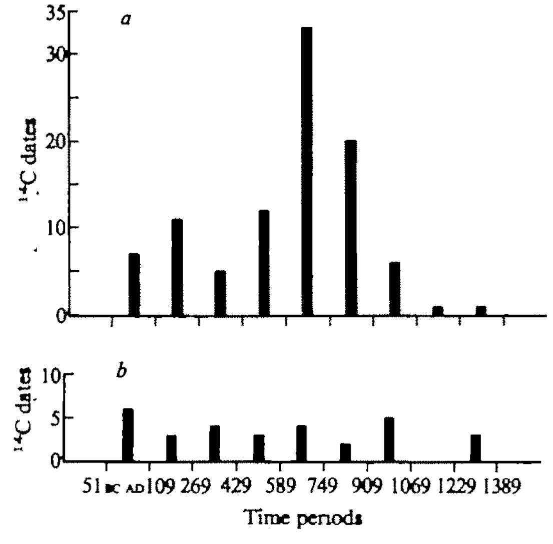

Fig. 3 *a*, Radiocarbon dates from elite contexts in the Southern Lowlands per time period; *b*, Radiocarbon dates from commoner contexts in the Southern Lowlands per time period.

calibration can be based on the BP radiocarbon date in Table 1. For some samples we have assigned a time range or several date possibilities instead of a single date. This situation exists because the calibration curve is not always singularly definitive. In plotting these cases on Fig. 3*a* we used either the midpoint of the range or the average of alternate dates. Table 1 also specifies the material composition of the radiocarbon sample, the laboratory number of the processed sample and the published reference.

Figure 3*a* indicates that Maya elite activities are present throughout the time span 51 BC to AD 589 (the Protoclassic and Early Classic periods) and their relative frequency shows only minor fluctuations. Elite activities dramatically increase during the period AD 589–749, and attain their zenith during this point. A significant decrease of these activities is observed during the period AD 749–909, followed by a drastic decline that takes place from AD 909–1069. Maya elite culture in the Lowlands during the period AD 1069–1389 is almost negligible.

The asymmetry and steep downslope in Fig. 3*a* clearly demonstrate a breakdown of elite class culture during the tenth century in the Lowlands, and its cessation, for all practical purposes, by AD 1069. The decline curves presented in Figs 2 and 3*a* are remarkably similar. A minor difference is that elite culture declines somewhat later than does the stela cult, a lag of the order of about one century. This was also noted by Hammond at Pusilha, Belize[18]. However, as Fig. 3*a* involves a broader spectrum of individuals and activities than Fig. 2, this time lag is expected. It is reasonable to conclude that the graphs in Figs 2 and 3*a* are measuring somewhat different aspects of the same phenomena—the rise and fall of the Classic Lowland Maya elite class. The similarity of the two graphs helps to establish the reliability of the ^{14}C frequency-distribution bar-graph method.

As previously discussed the depopulation rate of the Lowland centres, following the collapse of elite culture, is the most difficult problem of the Maya collapse. Figure 3*b* shows the distribution frequency of 30 ^{14}C dates, within the span 51 BC to AD 1389, that are from what may be called Southern Lowland Maya commoner contexts. In other words they are not inherently associated with elite manifestations. The ^{14}C samples come from commoner contexts that include house mounds, burials, middens, ridged fields, and lake sediment deposits which contain maize pollen as evidence of nearby agriculture.

It is immediately apparent that Fig. 3*b* indicates mildly fluctuating population cycles in the Lowlands, but no radical growth spurts or drastic declines. A slight population decline

Table 1 Lowland Maya radiocarbon dates for the span 51 BC to AD 1389

Site	Provenance	Context (elite or commoner)	Tree-ring calibrated date† (AD)	Radiocarbon date‡ (yr BP)	Material	Laboratory no	Ref
Mexico							
Aguacatal	Pit JA-1	C	300	1,645±140	Charcoal	I-1068	22
	Tr B-6, Level II	C	300	1,695±200	Animal bone	I-1069	22
	Tr B-6, Level III	C	700, 800, 900	1,212±170	Animal bone	I-1070	22
Balankanche Cave*	Chamber 2 urn	E	900–1,000	1,072±51	Charcoal	P-1132	23
	Chamber 2 hearth	E	1,000	1,028±42	Charcoal	P-1133	23
Batebatón*	Urn cremation	E	1,450	410±70	Charcoal	GRN-790	24
Becan*	Str IV, Rm 9	E	700, 800–900	1,230±95	Charcoal	I-4286	25
*	Str IV, Rm 8	E	650	1,295±95	Charcoal	I-4287	25
*	Str XXV	E	650	1,280±95	Wood	I-5028	25
*	Str XXV-sub	E	150	1,830±95	Charcoal	I-5029	25
*	Str X	E	650	1,345±95	Wood	I-5030	25
Chicanna*	Str II, Rm 1	E	650	1,280±95	Charcoal	I-5024	25
*	Str II, Rm 1	E	650	1,265±95	Wood	I-5025	25
*	Str XX	E	650	1,380±95	Wood	I-5026	25
*	Str XX	E	700, 800–900	1,230±95	Wood	I-5028	25
Chichen Itza*	Chultun by Cenote, Secondary burials	E	900	1,135±60	Bark	UCLA-1706	—
*	La Iglesia	E	900	1,170±70	Wood	TBN-313-1	26
†	La Iglesia	E	600	1,350±70	Wood	TBN-313-2	26
*	Red house	E	600	1,340±60	Wood	TBN-313-3	26
*	El Castillo lintel	E	900	1,160±70	Wood	Y-626	27
Chinkultic	Temple-platform tomb	E	1,000	990±70	Bone collagen	UCLA-1617	—
Dzibilchaltun*	Str 1 lintel	E	500–550	1,450±200	Wood	W-707	28
*	Str 612 hearth	C(?)	500	1,520±200	Charcoal	LJ-531	28
El Tigre	Ridged field	C	350	1,670±49	Wood	P-1625	29
Mayapan*	Str Q-162	E	1,250	700±95	Charcoal	GRO-452	30
*	Str R-87	E(?)	1,450	400±55	Burnt wood	GRO-1166	31
Palenque	South of palace	E	650	1,390±100	Charcoal	H-396	32
	Above bedrock, Ex 23	E(?)	550–650	1,440±100	Charcoal	H-397	32
	Ofrenda, 2, T-V, N group	E	450	1,565±105	Charcoal	H-639	32
	North side of palace	E	450	1 540±105	Charcoal	H-641	32
Sta. Rosa	Md B	E	150	1,845±50	Charcoal	GRN-1916	24
	Md F, Fl 3	C	100 BC–AD 50	1,990±65	Charcoal	GRN-1932	24
Sayil*	Main palace	E	950	1,100±70	Wood	Wis-144	33
*	Main palace	E	700, 800–900	1,230±60	Wood	IVIC-484	34
Tulum*	Str 21, Rms D, E	E	1,150–1,250	880±60	Wood	Y-393	27
Uxmal*	Structure behind magician pyramid	E	700, 800, 900	1,210±60	Wood	IVIC-485	34
*	Magician pyramid	E	650	1,390±50	Wood	Y-627	27
*	Nunnery quadrangle	E	900–1,000	1,065±100	Wood	GRO-613	30
Chiapas/North Yucatan?	Grolier codex	E	1,250	720±130	Bark paper	I-6107	35
Mexico–Guatemala?	Sculpture of Maya noble	E	550–650	1,425±120	Wood	I-490	36
Guatemala							
Altar de Sacrificios	House mound 33	C	350	1,290±155	Charcoal	GX-166	37
	Str A-II, fill	E	100 BC–AD 50	1,990±130	Charcoal	GX-169	37
	Str. A-III, burial 4	E	150	1,845±110	Charcoal	GX-168	37
	Str B-II	E	50	1,965±225	Charred beams	GX-171	37
	Str A-XIX, refuse	E	650	1,275±115	Charcoal	GX-170	37
Kinal	Main plaza, E temple	E	650	1,320±73	Wood	UM-124	38
Lake Petenxil	Pollen core from under 4 m of water	C	650	1,305±140	Gyttja	Y-988	39
	Pollen core from under 4 m of water	C	1,000	1,040±200	Gyttja	Y-1289	39
	Pollen core from under 4 m of water	C	1,350	635±140	Gyttja	Y-987	39
San Felipe	Pit F	C	50 BC–AD 50	1,970±100	Charcoal	Y-2416	40
	Md II, Cut 8	C	650	1,310±100	Charcoal	Y-2418	40
	Md II, Cut 6	C	550–650	1,430±100	Charcoal	Y-2419	40
Seibal	Burial 19	E	500	1,490±320	Collagen	UCLA-1640D	41
	Burial 17	E	550–650	1,420±95	Collagen	UCLA-1640C	41
	Mass burial 4	E	950	1,100±100	Burnt bone	UCLA-1640F	41
	Burial 12	C	1,000	1,000±65	Collagen	UCLA-1640A	41
	Burial 13	C	1,050	940±70	Collagen	UCLA-1640B	41
Tikal	T-II	E	650	1,365±50	Wood	P-960A	—
	T-II	E	450	1,605±51	Wood	P-961	—
	T-III V B	E	650	1,358±39	Wood	P-949	—
	T-III V B	E	650	1,352±45	Wood	P-950	—
	T-V	E	650	1,267±51	Wood	P-947	—
	T-V	E	650	1,317±51	Wood	P-948	—

Table 1 Lowland Maya radiocarbon dates for the span 51 BC to AD 1389 (*cont*)

Site	Provenience	Context (elite or commoner)	Tree-ring calibrated date† (AD)	Radiocarbon date‡ (yr BP)	Material	Laboratory no	Ref.
	T-VI B	E	500	1,527±51	Wood	P-962	—
	T-VI B	E	650	1,297±44	Wood	P-963	—
	T-VI B	E	650	1,364±50	Wood	P-1196	—
	T-VI B	E	1,000	999±44	Wood	P-1197	—
	T-VI B	E	650	1,372±49	Wood	P-1198	—
	T-VI B	E	650	1,308±47	Wood	P-1199	—
	T-VI B	E	900	1,125±46	Wood	P-1200	—
	T-VI B	E	650	1,398±42	Wood	P-1201	—
	Str 3D-43	E	650	1,290±47	Wood	P-964	—
	Str 3D-43	E	500	1,544±41	Wood	P-965	—
	Str. 5D-46	E	250	1,718±45	Wood	P-946	—
	Str. 5D-46	E	450	1,580±50	Wood	P-955	—
	Str. 5D-46	E	650	1,376±53	Wood	P-956	—
	Str. 5D-46	E	200	1,789±33	Wood	P-957	—
	Str 5D-46	E	450	1,555±46	Wood	P-958	—
	Str. 5D-46	E	300	1,677±46	Wood	P-959-A	—
	Str 5D-52, Rm. A, V.B 8	E	650	1,335±40	Wood	UCLA-158A	42
	Str. 5D-52, Rm. A, V.B. 9	E	650	1,304±60	Wood	UCLA-158B	42
	Str 5D-52, Rm A, V.B. 10	E	650	1,366±60	Wood	UCLA-158C	42
	Str. 5D-52, L 34, *a*	E	650	1,318±60	Wood	UCLA-158D	42
	Str 5D-52, L 34, *b*	E	650	1,303±50	Wood	UCLA-158E	42
	Str 5D-23, V.B. 4	E	650	1,350±52	Wood	P-294	43
	Str 5D-23, V.B 8	E	250	1,740±50	Wood	P-295	43
	Str. 5D-23, V.B 14	E	250	1,720±61	Wood	P-296	43
	T-IV Rm 2, V.B. 1	E	900	1,150±44	Wood	P-235	43
	T-IV Rm 2, V.B 2	E	700, 800–850	1,250±48	Wood	P-236	43
	T-IV Rm 2, V.B. 3	E	700, 800–850	1,260±72	Wood	P-237	43
	T-IV Rm 2, V.B. 4	E	700, 800, 900	1,200±48	Wood	P-238	43
	T-IV Rm 3, V.B 1	E	700, 800–900	1,230±48	Wood	P-242	43
	T-IV Rm 3, V B. 2	E	700, 800, 900	1,220±55	Wood	P-243	43
	T-IV Rm 3, V B 3	E	700, 800, 900	1,210±83	Wood	P-244	43
	T-IV Rm 3, V B 4	E	700–750, 900	1,180±79	Wood	P-245	43
	T-IV L 3, *a*, *d*, or *f*	E	700, 800, 900	1,190±47	Wood	P-248	43
	T-IV L 3, *e* or *g*	E	700, 800, 900	1,210±48	Wood	P-249	43
	T-I Rm 1, V B. 1	E	500	1,490±85	Wood	P-229	43
	T-I Rm. 1, V B 2	E	650	1,300±93	Wood	P-230	43
	T-I Rm 2, V B 2	E	700, 800–850	1,260±49	Wood	P-232	43
	T-I Rm 3, V B. 2	E	700, 800, 850	1,260±56	Wood	P-234	43
	T-I L 3, *c* or *d*	E	650	1,270±49	Wood	P-247	43
	T-I L 3, *e*	E	700, 800–850	1,260±110	Wood	P-251	43
	Str 5D-34, Rm 3, C-19	E	900	1,150±47	Charcoal	P-278	43
	Str. 5D-34, Rm 3	E	900	1,180±55	Charcoal	P-279	43
	Str. 5D-34, Rm 3, Altar	E	500	1,530±52	Charcoal	P-281	43
	Str. 5D-34, Construction fill	E	450	1,550±58	Charcoal	P-284	43
	Great Plaza Chultun	E	50 BC–AD 50	1,970±44	Charcoal	P-285	43
	Quarry 5D-1	C(?)	100 BC–AD 50	2,000±63	Charcoal	P-287	43
	N Acropolis earth layer fill	C	50	1,930±51	Charcoal	P-757	44
	N Acropolis, Burial 85	E	50	1,934±63	Wood	P-535	44
	N. Acropolis, Str. Sub. 2-1st	E	50	1,951±46	Charcoal	P-561	44
	N Acropolis, in situ pit	E	100	1,874±54	Charcoal	P-562	44
	N Acropolis, Ceremonial debris	E	50	1,926±48	Sherds, ash	P-565	44
	N Acropolis, Burial 125	E	250	1,777±45	Charcoal	P-768	44
	N Acropolis, Str 5D-26-1st	E	400	1,625±60	Wood	P-571	44
	N. Acropolis, Str 5D-20-2nd	E	650	1,308±49	Charcoal	P-569	44
	N. Acropolis, Str. 5D-20-1st	E	650	1,274±50	Wood	P-570	44
	N Acropolis, Str 5D-26-1st	E	400	1,624±61	Charcoal, sherds	P-572	44
	N. Acropolis, Burial 22	E	550, 650	1,416±52	Charcoal	P-573	44
	N Acropolis, Str 5D-26-1st	E	650	1,285±52	Charcoal	P-574	44
	N Acropolis, Hearth	E	250	1,749±59	Charcoal, bone	P-575	44
	T-IV L 3, left plank	E	700, 800–850	1,250±50	Wood	UCLA-1373A	45
	T-IV L 3, 3rd plank from left	E	650	1,370±50	Wood	UCLA-1373B	45
	T-IV L 3, right plank	E	650	1,400±60	Wood	UCLA-1373C	45
Uaxactun	Str. A-VIII, Rm. 8	E	250	1,780±60	Wood	Y-367	27
	Str. A-V, Rms 36, 54	E	650	1,330±70	Wood	Y-368	27
Belize							
Altun Ha	Str. E-1 tomb	E	500	1,544±62	Carbonized copal?	P-1028	44
Aventura	Ex. 2-3, Midden	C	500–550	1,450±40	Charcoal	UCLA-1921E	5
	Ex 2-3, Midden	C	1,250	680±80	Shell	UCLA-1938E	5
	Ex 1-1, Midden	C	650	1,400±80	Shell	UCLA-1938B	5
	Ex 3-2, Midden	C	1,000	1,025±80	Shell	UCLA-1938F	5
Caledonia	Ex. 20-13, Midden	E	300	1,680±70	Charcoal	UCLA-1921F	5
Chan Chen	Ex 24-3, Habitation soil	C	100	1,865±55	Charcoal	UCLA-1921B	5
	Ex. 30-5, Str B-3	E	300–450	1,580±60	Charcoal	UCLA-1921C	5
	Ex. 28-2, Construction fill of Str. F-1	E	200–300	1,770±50	Charcoal	UCLA-1921D	5
Cuello	Op. 17B, Level 23	C	250	1,700±60	Burnt wood	UCLA-1985D	46
	Op 17B, Levels, 20, 22	C	900	1,140±100	Burnt wood	UCLA-1985BC	46
	Op 17G, Level 12	C	1,050	930±85	Burnt wood	UCLA-1985F	46
Las Cuevas	Outer cave, Tr 7	E	1,050	920±150	Charcoal	BM-37	47
Patchchacan	Ex 14-2, Burial	C	1,250	760±110	Collagen	UCLA-1938C	5
Rio Frio Cave	West side of far cave	E	900	1,120±150	Charcoal	BM-67	48
San Jose	Main Palace	E	1,000	1,010±50	Wood	UM-122	38
Tiger Bay Cave	Upper chamber, torch	E	1,200	780±60	Wood	UCLA-1935A	—
Honduras							
Copan	Hieroglyphic stairway construction fill	E	250	1,700±110	Charcoal	UCLA-1419	49
	Hieroglyphic stairway	E	700, 800, 900	1,200±70	Charcoal	UCLA-1420	49
La Cinteada	Plaza 1, Pit 6	C(?)	250	1,700±125	Burnt wood	UCLA-1992B	—
	Plaza 2, Str. 5, Pit 7	E	900–1,000	1,050±220	Burnt wood	UCLA-1992C	—
Los Naranjos	Habitation soil	C	100	1,850±100	Soil	GIF-1324	50
	Habitation soil, 2 3 m depth	C	250	1,700±100	Soil	GIF-1473	50
	Habitation site, hearth	C	500	1,530±100	Soil	GIF-1472	50
	Habitation soil	C	500	1,500±100	Soil	GIF-1474	50
	Soil of ceremonial area	E	700, 800–850	1,260±90	Soil	GIF-1326	50

* The radiocarbon date is not plotted in Fig. 3*a*, *b*, either because it is in the Northern Lowlands or it falls outside the range 51 BC to AD 1389.

† Date is rounded off to nearest 50

‡ Half life 5,568±30 yr.

might be indicated for the period AD 1069–1389, as only three ^{14}C dates are present, but given the overall small sample size this is hardly evidence of a major demographic catastrophe for this period. Figure 3*b* does not establish, then, a depopulation curve similar to those previously demonstrated for the cessation of the stela cult and general elite activities during the Late Classic–Terminal Classic periods.

The lack of a collapse curve in Fig. 3*b* may be due to the relatively small sample size or perhaps indicate a historical fact. In particular, the sample used is three times smaller than that in Fig. 3*a* and about 12 times as small as that in Fig. 2. A further complication is the geographical context of the ^{14}C samples. Only 35% of the dates come from the Peten, while the rest are from adjacent areas such as Belize, Chiapas, and Honduras, areas that may have been the scene of a delayed collapse[4]. Unfortunately the 30 dates represent most, if not all, of the ^{14}C dates presently available from Lowland commoner contexts. We therefore urge Maya archaeologists to obtain more commoner-associated ^{14}C dates, as these dates should eventually be of great value in resolving the depopulation issue and be more representative of the cultural situation as it actually was. Yet we also wonder if any major depopulation would not have been apparent in the 30 dates obtained so far.

A critical reexamination of the depopulation problem has in fact been offered by J. E. S. Thompson[19] who concluded that wholesale desertion of the Central area at the close of the Classic period can be ruled out. He proposed that a Postclassic population, probably reduced in size, had continued to exist in the Central Lowlands in isolated villages. This population was depopulated, in turn, during the first 50 yr of contact by Europeans, due largely to newly-introduced diseases such as dysentery, hookworm, and malaria. Additional support for a rethinking of the depopulation issue is found in Hellmuth's bibliography of the post-Conquest Lowland Maya[2] and in a recent article by Adams and Smith that compares the extensive population decline in mediaeval Europe to that of the Late Classic Maya[21].

The decline curves for the cessation of the stela cult and the breakdown of general Late Classic elite activities seem to be very similar, although the latter process continued for another century following the demise of the stela cult. The ^{14}C bar-graph that simulates the population level of the commoner class during this period does not, however, indicate the occurrence of a major demographic catastrophe.

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Normal human tissues contain RNA and antigens related to infectious adenovirus type 2

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Normal human tissues have been found to contain RNA which hybridises with four regions of the adenovirus type 2 genome, including one which contains the transforming gene(s) of this virus. The RNAs have also been extracted from gorilla organs, but not from those of chickens, suggesting that they are a feature of all normal higher primate tissues. Serological tests suggest that these homologous RNAs are translated into proteins connected with essentially normal and virus-infected cell functions.

THE adenoviruses[1,2] are associated with respiratory infections in humans. Many serotypes of these viruses can induce tumours in rodents[3–6], and adenovirus type 5 transforms human cells in culture[7]. During studies on human cancer, we have investigated the possibility that human tissues might contain RNA sequences related to human DNA viruses, using the technique of *in situ* hybridisation[8–10]. We have found that normal human tissues contain RNA that hybridises with four regions of the genome of adenovirus type 2 (Ad-2). One of these regions includes the transforming gene(s) of this virus[7,11,12]. This suggests either that genes similar to viral transformation genes are present in man in

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much the same way that the avian *sarc* genes are homologous in base sequence to the *src* (transforming) gene of the Rous sarcoma virus[13,14], or that infectiously acquired Ad-2 DNA can persist under host control. The human cells expressing the RNAs in greatest abundance seem to be solitary, possibly circulating cells. Human tissue cultured cells apparently do not contain an abundance of these RNAs, which may account for the fact that they have not been described previously.

Hybridisation studies

In looking for possible RNA sequences related to Ad-2 viral DNA in uninfected human organs, we initially used placenta as the RNA source because it is available in quantity. Moreover, fetal tissues are often associated with endogenous viral sequence expression[15–17]. Total RNA was extracted by standard procedures[18] and iodinated[19] with ^{125}I for use in hybridisation. Equal amounts of Ad-2, human DNA and λ phage DNA or Ad-12 DNA were denatured and spotted on nitrocellulose strips and hybridised by the usual procedure[20] (for details see legend to Fig. 1). *Escherichia coli* total ^{125}I-labelled RNA served as an RNA control, hybridised to a parallel series of spots but substituting *E. coli* DNA for phage DNA. The hybridised strips were then treated with RNase and, after suitable washing, were dried and autoradiographed on presensitised X-ray film (Royal X-omat XHI Kodak) at −70 °C in cassettes fitted with intensifying screens. Such spot tests are extremely sensitive and the amount of signal due to hybridisation or to background is immediately visually apparent. Hybridisation can be quantitated if necessary by cutting out the spots and scintillation counting. This procedure was followed as described later to construct melting-temperature curves of the hybrids.

After autoradiographic exposure, there was no trace of hybridisation to phage DNA. In fact, the spots containing this DNA were, if anything, below background radioactivity levels in some cases, suggesting that the bound DNA may have preempted potential binding sites for molecules that contribute background. There was, as expected, very strong hybridisation to human DNA. Ad-2 DNA showed moderate hybridisation (Fig. 1*a*), which easily reached autoradiographic saturation on prolonged exposure (Fig. 1*b*). Ad-12 DNA showed a barely detectable autoradiograph (not shown). *E. coli* ^{125}I-labelled RNA did not hybridise with any of these DNAs, but did so with *E. coli* DNA.

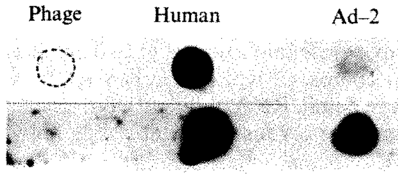

Fig. 1 Spot tests of hybridisation of ^{125}I-labelled human placental RNA to DNA of λ phage, human and adenovirus type 2 (Ad-2). The location of the negative hybridisation is shown by a dotted circle. Hybridisation has occurred to human DNA and to a lesser, but definite, extent to Ad-2 DNA. The conditions for hybridisation were as follows. The DNA samples were brought to the same concentrations, and 1-μg aliquots bound in spots to a strip of nitrocellulose filter by the method of Gillespie and Spiegelman[20]. The filter strip was then incubated for 6 h at 68 °C in 6 × SSC containing 0.02% each of Ficoll, bovine serum albumin (BSA) and polyvinyl pyrollidone (PVP). It was then transferred to 3.6 ml of this same solution containing 2 mM, EDTA, 0.5% SDS and ~7×10^7 c.p.m. of placental total RNA previously prepared[18] and radiolabelled[19] with ^{125}I. The mixture was annealed at 68 °C for 15–16 h, cooled rapidly and the filter strip washed in 2 × SSC, treated with RNase (40 μg ml^{-1}, 1 h, 37 °C in 2 × SSC), washed in 2 × SSC, treated with pronase (400 μg ml^{-1} in 2 × SSC, 1 h), incubated in 2 × SSC, 0.5% SDS for 6 h at 68 °C, washed exhaustively in a large volume of 2 × SSC, dried in a vacuum oven and autoradiographed for (*a*) 10 d, (*b*) 3 weeks at −70 °C using Kodak Royal X-omat XHI X-ray film presensitised by brief exposure to light from a calibrated masked flash gun.

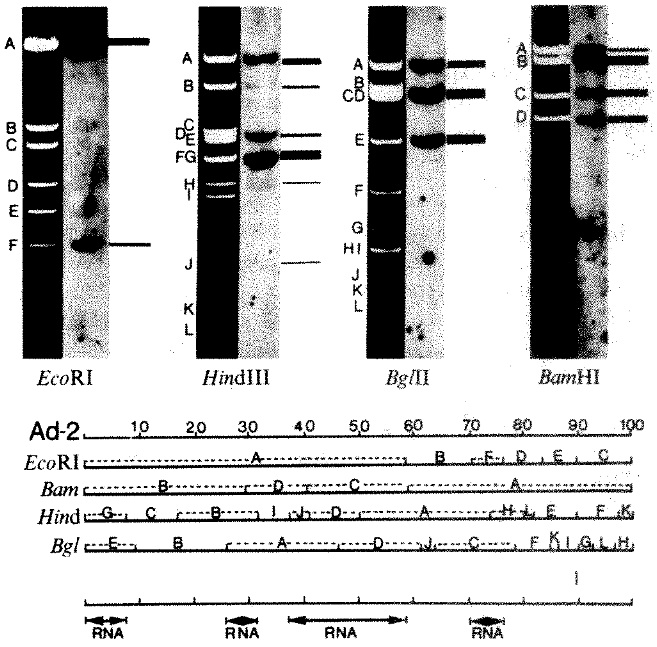

Fig. 2 Restriction digests of Ad-2 separated into fragment classes by electrophoresis on agarose gels stained with ethidium bromide and photographed in ultraviolet light, are shown paired with autoradiographs of ^{125}I-labelled placental RNA hybridised to the same fragments after transfer to nitrocellulose filter membrane and hybridisation as detailed in the legend to Fig. 1, and in the text. The bands in the digests are identified by letters to the left of each gel track. For technical reasons, the smallest restriction fragments do not photograph satisfactorily, and some of the minor autoradiographic bands are difficult to detect consistently together in the same autoradiograph. Because of this we have included a third track which indicates, diagrammatically, for each enzyme digest, all the bands to which we have obtained hybridisation in replicate experiments. These data are also shown below the photographs in the form of maps of the positions of the restriction fragments obtained with each enzyme in relation to the Ad-2 DNA map, which is divided into 100 fractions, according to the usual convention. The restriction fragments which hybridise with placental RNA are indicated for each digest by dotted lines over the fragment concerned. From these data, the provisional assignments have been made for the maximum limits to the Ad-2 genome sequences which hybridise with human total RNA; these are represented as double-ended arrows below the relevant locations. The conditions for electrophoresis were 1% agarose gels run at 40 V for 10 h, or 80 V for 4 h, in a buffer containing 15 mM Tris, 18 mM NaH_2PO_4, 0.5 mM EDTA, *p*H 7.8.

These results indicated that Ad-2 DNA contained some sequences homologous with human RNA, although they did not show whether these might have arisen from possible contaminating host cell sequences. Nevertheless, the fact that Ad-2 and Ad-12 were grown in similar human cells but behaved differently on hybridisation with human RNA suggested that contamination was unlikely. To investigate this point, we hybridised the same DNAs after digestion with the restriction endonuclease *Eco*RI, which cuts λ and adenovirus DNAs into small numbers of fragments; these can then be separated on agarose gels and transferred to nitrocellulose strips by the Southern procedure[21] for subsequent hybridisation with the same ^{125}I-labelled human RNA probe. After autoradiographic exposure there was hybridisation only to Ad-2 DNA and this was limited to the *Eco*RI A and F fragments (Fig. 2). Separate lots of Ad-2 DNA were then cut with three further restriction endonucleases, *Bam*HI, *Bgl*II and *Hin*dIII, which are known to produce genomal fragments overlapping and different from those produced by *Eco*RI. Hybridisation of human placental ^{125}I-RNA to these sets of fragments, as before, yielded the following hybridisation data (Fig. 2).

The fragments that hybridised were *Eco*RI A and F; *Hin*dIII A, B, D, FG, J and H (the latter two very faintly); *Bgl*II A, CD and E; and *Bam*HI A, B, C and D. These data define the following provisional maximum limits for the hybridising regions. As only the A and F fragments of *Eco*RI hybridised, all the observed hybridisation must lie within these limits. As *Eco*RI A, *Bam*HI B, *Hin*dIII G and *Bgl*II E fragments hybridised, but *Hin*dIII C or *Bgl*II B did not, the left end of *Hin*dIII C defines the most rightward limit of the first region of hybridisation at 7.5 units on the Ad-2 map[22]. This region, from 0 to 7.5, is known to contain the transforming gene(s)[23]. Because the *Bgl*II B fragment did not hybridise, but the *Bgl*II A fragment did, the latter, with the other restriction enzyme fragments that overlap it, defines the beginning (left end) of the next region of hybridisation at approximately 26 units. *Hin*dIII I did not hybridise but *Hin*dIII J did, so the I fragment defines a non-hybridising gap, and the left end of J defines the beginning of the next region of hybridisation at approximately 37 map units, extending to approximately 58.5 units, which is defined by the left end of *Eco*RI B, which did not hybridise. By the same reasoning the *Eco*RI F fragment must define the limits of the fourth region of homology as between approximately 70.7 and 75.9 map units. From this analysis, we presume that the doublet *Hin*dIII FG concerns hybridisation only to the G fragment, but that hybridisation occurs to both fragments of the *Bgl*II CD doublet. This point can be finally decided when a more complete analysis of the hybridisation patterns has been carried out. This provisional map summarising the maximum limits within which the hybridising regions lie is given diagrammatically in Fig. 2.

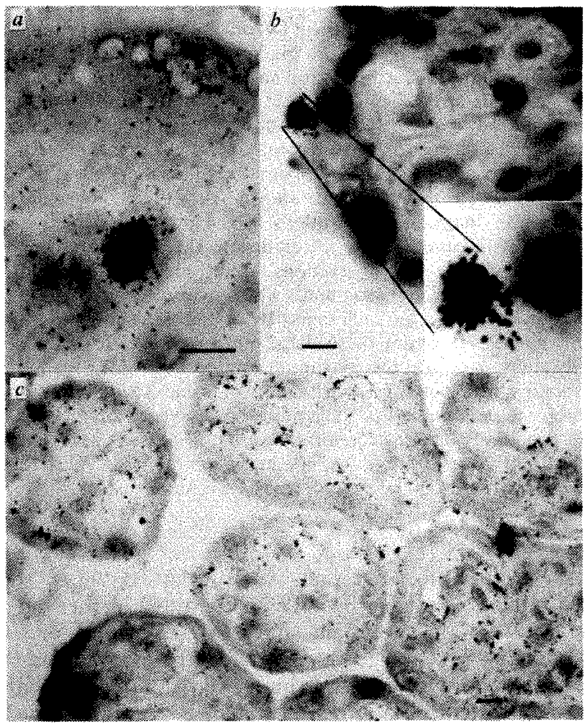

Fig. 3 Autoradiographs showing concentrations of Ad-2 complementary RNA in placenta. Ad-2 DNA labelled by the 'nick-translation' reaction with ^{3}H, was denatured and hybridised *in situ* to frozen sectioned human placenta in conditions that detect complementary cellular RNA[8–10]. *a*, A 'solitary' cell that shows a high concentration of label signifying a high concentration of Ad-2-related RNA. This cell type occurs relatively infrequently (about 2–3 per ×40 objective field) and occasionally seems to be loosely attached to the tissue (*b*). This may reflect the fact that it is a circulating cell. *c*, An example of a generally labelled area of placenta, after hybridisation as above. The grain distribution in this autoradiograph suggests that whole areas of this tissue contain Ad-2-related RNA at a lower level than the solitary, highly labelled, cells shown in *a* and *b*. Autoradiographic exposure 3 weeks. Controls of similarly hybridised λ phage DNA were negative. Scale bars, 10 μm.

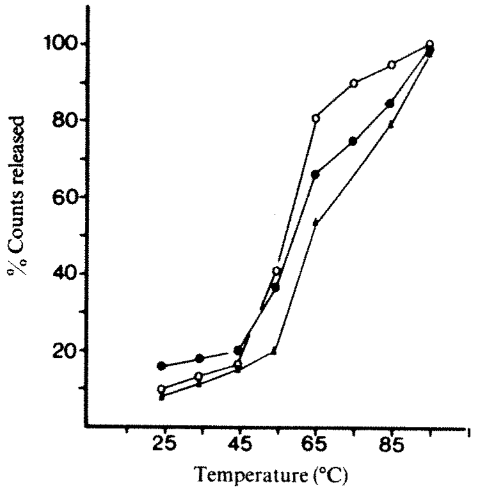

Fig. 4 Thermal dissociation of hybrids formed between human total ^{125}I-labelled RNA and human total DNA (○), and Ad-2 whole genomal DNA (●, sample 1; ▲, sample 2), from different sources. The curves show similar features, with both Ad-2 DNA samples having formed hybrids of rather higher thermal stability than total human DNA. Hybridisation conditions are described in the legend to Fig. 1. Dissociation was carried out by cutting spots of hybridised DNA from nitrocellulose filters of spot tests that had been shown to be positive by previous autoradiography on X-ray film as in Fig. 1. These were then counted by scintillation spectrometry in toluene-based scintillation fluid, washed and then treated in diethyl pyrocarbonate 0.1 in SSC (0.15 M NaCl, 0.015 M sodium citrate, *p*H 7.4) for 30 min, washed in SSC, and heated for 10 min in 1 ml 0.1 SSC and 0.1% SDS at each of the temperatures shown. The counts released were recovered from solution by trichloroacetic acid precipitation in the presence of 100 μg BSA per sample, dried by suction filtration and counted by scintillation spectrometry. Approximately 80% of the original radioactivity was recovered.

This provisional map has been confirmed with a further two independently obtained samples of Ad-2 DNA and with 10 separate samples of placentas, obtained from normal births, from two hospitals. We have also found the same pattern in the case of human adult liver RNA, and with liver, kidney, brain and spleen RNA obtained from a gorilla which died from the effects of minor surgery. The RNAs from four separate human cell lines maintained in permanent culture gave very faint patterns of hybridisation compared with the organ RNAs and are being studied in more detail. RNA obtained from chickens in the same manner as for other organs of primates did not hybridise in two separate tests.

That human organ RNA and other higher primate RNA contain sequences with homology to certain regions of the genome of Ad-2, but that such RNA apparently is relatively much less abundant in cell lines of human origin, indicated that the distribution of cells having such an abundance was not uniform. We investigated this aspect by hybridising ^{3}H nick-translated, denatured Ad-2 DNA to human placental cryostat sections *in situ* using a method which permits hybridisation to mRNAs[8–10]. After S_1 nuclease treatment and thermal rinsing to remove unhybridised probe or poorly matched hybrids, the preparations were autoradiographed and examined microscopically. Strong hybridisation occurred only to certain cells in the sections. These seemed to be solitary cells, with hybridisation to almost the entire area of the cell (Fig. 3*a*, *b*). Other areas of the sections showed a lower level of hybridisation which was, however, above the non-cellular background (Fig. 3*c*). There was no hybridisation in the case of λ phage ^{3}H-DNA hybridised in identical conditions. We presume from this that the bulk of the RNA that hybridised is contributed by a tissue cell subpopulation and that cells of this type are not among the cell lines that we examined.

Melting-temperature experiments

We carried out melting-temperature experiments to determine the thermal stability of the the hybrids formed on nitrocellulose between Ad-2 DNA and placental RNA. These were done by cutting out the relevant hybridised spots from the filter and heating them in solution at increasing temperature (see legend to Fig. 4) to measure the loss of hybridised RNA from the filters. Hybrids formed between human ^{125}I-labelled total RNA and two separate Ad-2 DNA samples and with total human DNA were compared. As can be seen from Fig. 4, the melts of Ad-2 hybrids showed similar characteristics and similar, though slightly higher, T_m values compared to the homologous hybrids, indicating that the hybrids to Ad-2 were reasonably thermally stable. This result was expected from the annealing conditions (legend to Fig. 1) which were quite stringent and essentially identical to those used in most published Ad-2 hybridisation studies (see discussion). We conclude that the hybrids that we detect between human RNA and Ad-2 DNA are at least as well matched as those in previous transcription studies of Ad-2. We are now investigating methods to determine the precise lengths of the hybridised regions. However, this is complicated by the fact that the organ RNA is to some extent degraded.

Immunological studies

Hybridisation between a viral DNA and human RNA from apparently uninfected tissues suggests similar protein coding functions in the sequences concerned. To test this hypothesis, rabbit cells infected with a clinical isolate of Ad-2 were used to raise antisera separately against the early-infected and late-infected cells (see legend to Fig. 5). The antisera were then reacted with six human cell lines (not shown) in an indirect immunofluorescence assay. All except one cell type, a human glioma (NG-118H) transformed by Rous sarcoma virus which was faintly positive, were completely negative. Controls of Ad-2-infected HEp 2 cells were brilliantly positive (Fig. 5*a*) as were transformed rat cells containing the extreme left-hand fragment of the Ad-2 genome[12] (not shown). Placental sections were also positive when treated with the anti-early antisera (Fig. 5*b*,*c*), but not with anti-late-infected cell antiserum. The fluorescence was concentrated on the surfaces of cells lining what seemed to be channels in the tissue as well as occurring diffusely in the cytoplasm of many areas. Some cells showed the nuclear fluorescence usually seen in transformed cells. However, this was rare and not intense. Of 10 placentas tested, 8 were undoubtedly positive in these tests and 2 were judged negative. We do not understand this variation, as all were positive in hybridisation tests for Ad-2-related RNA; however, it may be due to the fact that the antigens concerned are unstable and that the variations reflected the variable storage conditions of the tissues before we obtained them.

Antigens were then solubilised[24] from placental tissue and tested by gel diffusion in Ouchterlony plates against both early- and late-infected cell antisera, using controls of Ad-2-infected rabbit cell antigens (Fig. 6). There was no reaction with any dilution of uninfected cell antigens (not shown). Strong precipitin lines formed between the early-infected cell antisera and the antigens of both early- and late-infected cells, as expected, for both will contain the same early antigens, although presumably in different concentrations. Weaker but quite definite precipitin lines formed between this same antiserum and the wells containing placental antigens, and these showed partial identity with one of the lines originating from the early-infected cell antigen wells but no identity with late-infection antigens. The tests against anti-late-infected cell antisera were even more strongly positive against the infected cell antigens, particularly the late-infected cells. However, there were no visible precipitin lines between this antiserum and the wells containing placental antigens. Separate tests were then carried out in which a range of dilutions of the placental antigens were tested against each antiserum separately. No dilution used gave any precipitin lines with the late-infected cell antiserum (not shown). The early-infected cell antiserum, however, showed a single precipitin line that altered position between the antigen and antibody well depending on concentration. This shift is due to antigen–antibody concentration which affects the solubility and hence the mobility of the antigen–antibody complex. The fact that there was no precipitin line in the case of anti-late-infected cell antiserum suggests that the concentration of the relevant antibodies to early-infected cell antigens was very low.

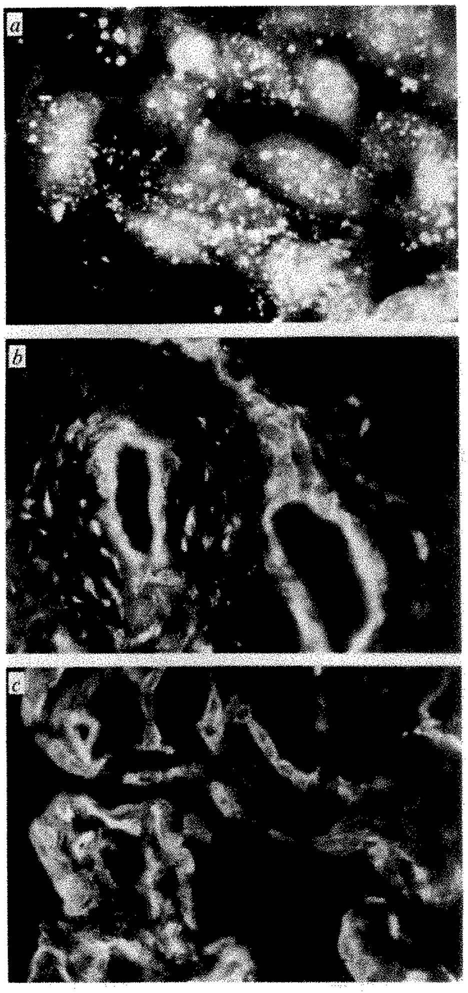

Fig. 5 Indirect immunofluorescence tests for antigens on: *a*, Ad-2 early-infected human cells (HEp 2); *b*, *c*, normal human placenta cryostat sections, using antiserum raised against Ad-2 early-infected rabbit cells (Rk-13), at a dilution of 1/40 and sheep anti-rabbit IgG conjugated with fluorescein isothiocyanate (Wellcome). The rabbit cells were grown in medium 199 supplemented with 2% rabbit serum and the antisera raised in rabbits. Cells were infected for 20 h in the presence of 20 μg ml^{-1} cytosine arabinoside. The resulting antiserum was negative for virus neutralising activity. Uninfected cell antiserum was also raised and was negative by immunofluorescence with these same subjects, and infected cell antiserum was negative with both uninfected HEp and RK-13 cells. The infecting adenovirus was obtained from a clinical isolate. Antiserum to late-infected cells was negative in similar tests. Fluorescence in the HEp cells is mainly on nuclear structures with some cytoplasmic fluorescence. Placental sections show stronger fluorescence in cytoplasmic regions, especially on what are apparently cells lining channels or vessels in the placenta, but nuclear fluorescence is also visible. These results, which demonstrate the presence of cross-reacting antigens between early-infected and apparently uninfected placenta, have also been obtained with all other tissues but not with cell lines tested (see text), and are confirmed by Ouchterlony gel diffusion (Fig. 6 and text).

We conclude from these preliminary immunological experiments that rabbit cells infected with Ad-2 contain certain antigens that are not present in uninfected rabbit, or human, cells in culture. We further conclude that some of the Ad-2-induced antigens are identical to tissue antigens present in most

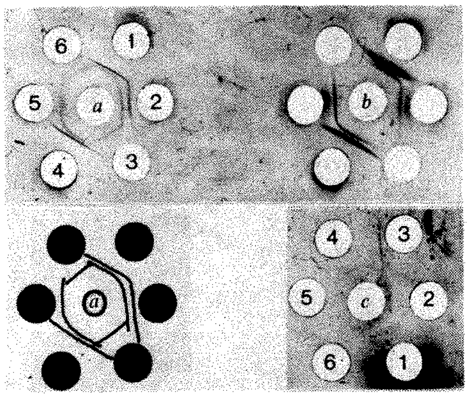

Fig. 6 Ouchterlony gels showing patterns of precipitation obtained between *a*, anti-early Ad-2-infected Rk-13 cells, and *b*, anti-late Ad-2-infected Rk-13 cell antisera. Wells are numbered 1–6 in the same manner in each case. Wells 1 and 4 contained dilutions of 1/50 and 1/100 of late Ad-2-infected antigens; 2 and 5 contained dilutions of 1/50 and 1/100 Ad-2 early infection antigens; 3 and 6 contained 1/50 and 1/100 dilutions of placental antigens. An interpretation of the pattern seen in *a* is shown below it. In *a*, precipitin lines can be seen between both wells containing placental antigens and the central well containing anti-Ad-2 early-infected Rk-13 cells. In 3 there is a suggestion of partial identity with one of the lines associated with well 2 as judged from the single spur. There are no lines visible between the central well and wells 3 and 6 in *b*, indicating absence from the late-infected cell of antigenic components related to those of normal placenta. Dilution experiments with this particular combination over the range of 1/10 to 1/1,000 of antigen concentration revealed no precipitation (not shown); however, a similar range of dilutions of the same combination in the case of *a* showed an arc of precipitation spiralling out from the centre well according to the dilution of antigen; this is shown in *c*, in which all wells contained placental antigen, as follows: (1) undiluted; (2) 1/10; (3) 1/50; (4) 1/100; (5) 1/1,000; (6) saline. These data confirm that normal human placentas express antigens that are associated with Ad-2 early infection as shown by immunofluorescence in Fig. 5.

human placenta samples, concerning mainly the early proteins of the adenovirus infectious cycle. Such antigens are not detectable on cell lines of human origin by immunofluorescence but were present in all tissues of gorilla (not shown). We are now attempting to characterise these antigens in more detail. The presence of antigens related to Ad-2 early function proteins in humans and in gorilla tissues correlates with the presence in these tissues of RNA molecules that hybridise with regions of the Ad-2 genome involved in coding early function proteins. We suppose from this that these RNAs code for the antigens that are shared between apparently normal human, and other primate, tissues. The *in situ* hybridisation result using Ad-2 DNA probes suggests that certain cells, or cells in a certain transitory state, which constitute a minor subpopulation in the placenta, contain a high concentration of the Ad-2-related RNA. The connection between this observation and the fact that the antigens concerned are rather ubiquitous in the tissues examined is unclear but is discussed below. However, as there are at least four regions of the Ad-2 genome that hybridise, it is possible that different cell types contain different proportions, or types, of the corresponding, homologous, RNAs, and consequently code for different antigenic moieties.

Discussion

The present findings are unique in the sense that there have been no previous reports of RNA sequences related to adenoviruses in apparently uninfected human cells. These findings are particularly intriguing as they relate to adenoviruses that are potent transforming viruses in animal hosts[3–6]. Moreover, the regions of homology include sequences in the extreme left-hand end of the Ad-2 genome that contains transforming genes of this virus. The consistency of the findings, there being no negative results in the 11 human tissue samples included in this study, indicates that the RNAs concerned may be ubiquitous in the population. Moreover, the presence of the same RNA molecules in gorilla is indicated from the similarity of the hybridisation patterns obtained with RNA from kidney, liver, spleen and brain. This suggests that the RNAs may also be common within the higher primate group, although more examples, involving some from a natural population, will be essential to support this.

Our criteria for hybridisation of the RNAs to Ad-2 DNA are those normally used in hybridisation mapping of Ad-2 (refs 25, 26) and are reasonably stringent taking the genome as a whole. Increasing the hybridisation temperature to 75°C reduced but did not prevent hybridisation. Moreover, the thermal stability of the Ad-2 DNA – human RNA hybrids seems representative of hybrids formed between human total DNA and the same human RNAs used in this work. We conclude from this that the hybrids are reasonably well matched. This is supported by the fact that there is demonstrable homology between the antigens coded during infection by Ad-2 and those present in human placenta and liver, and gorilla organs, which indicates similar coding functions of the RNA produced early in Ad-2 infection and that present in apparently normal higher primate organs.

These data, although consistent, are at present limited and the possible interpretations are, correspondingly, tentative. However, the findings are provocative and invite some speculations as to their significance. The RNAs concerned may have originated in either of two, not necessarily mutually exclusive, ways. Namely, host acquisition by an infectious process or the evolution of Ad-2 (and the C group adenoviruses) through the acquisition of nucleotide sequences similar to those found in the natural primate host.

The facts relevant to these interpretations are that only certain Ad-2 DNA regions are represented in human RNA. This tends to rule out a productive Ad-2 infection as the RNA source, as we would then expect to find RNA representative of the whole genome. Because all individuals investigated contained the RNAs in question, it is obvious that the mode of transmission is very efficient. This rules out a particular defective virus as the source unless we also assume that transmission is vertical and widespread. In this case, it is debatable whether such sequences belong to the host or to a virus. We cannot, however, exclude the possibility that the sequences we detect belong to a non-defective, incompletely Ad-2-related virus, in our view an unlikely explanation, or that Ad-2 DNA may be carried in the manner of Epstein–Barr virus[27], following infectious contact and subject to specific host transcriptional control.

The second hypothesis is that Ad-2 and related strains have evolved, or otherwise acquired, genes that are related to certain genes of their natural hosts. As it is known that Ad-5 can integrate with host DNA[28], such evolution seems likely. Also, RNA tumour viruses of chickens and mice contain sequences related to their host cells. In avian sarcoma viruses the transforming gene *src* is related in nucleotide sequence to the host gene *sarc*[13]. It is also known that the transforming region of a primate virus SV40, which is totally unrelated to Ad-2, is rather similar to Ad-2 in its transcriptional structure[29], although it is not known whether the proteins coded are functionally analogous. Such convergent evolution might reflect the fact that both viruses have acquired or evolved control elements concerned with the same host function, as both are primate viruses. Presumably, the advantage of such a mode of evolution would be that the virus would be better adapted to subvert host functions, for example, by using the host RNA splicing systems. Pressure to acquire control of the same system on the part of unrelated viruses could stem from the fact that the functions concerned are key elements in growth control. In this regard it is known that Ad-2 replicates in human cells significantly faster than Ad-12 (refs 30, 31), which does not, as far as we can detect, share the host-related nucleotide sequences.

Whether or not either hypothesis is wholly correct, our findings do indicate that human cells possess transcripts, from whatever source, closely related to DNA that maps within the transforming region of the adenovirus genome. This may be relevant not only to how the virus itself replicates and transforms, but also to how malignant human cells may alter their growth patterns. Moreover, at least four regions of homology are detectable which may mean that at least four different RNAs related to the viral DNA exist in human cells. In future studies these can be recovered and their corresponding genes cloned and identified. In the event that the second hypothesis is substantially correct, such genes may relate to important functions connected with cell and virus growth control. A search for similar sequences related to other DNA viruses is also suggested from the fact that antigenic similarity has been described between SV40-induced surface antigens and fetal antigens in hamsters[32].

The fact that Ad-2 early function antigens cross-react immunologically with apparently ubiquitous antigens present in the natural host, regardless of their origin, is likely to be medically significant. Bacterial pathogens that share antigens with human cells can precipitate autoimmune reactions, as in the rheumatic disease caused by *Streptococcus pyogenes* infections. Persistent adenovirus infections might therefore, by analogy, precipitate similar diseases. Thus, there might be a link between other rheumatic conditions or autoimmune diseases and the antigens that we detect. Of particular interest here is the identity of the cell type that seems to synthesise a high concentration of Ad-2-related RNA as determined by *in situ* hybridisation. If such synthesis implies a high content of related antigens, such a cell might be particularly at risk from an autoimmune reaction directed at these common antigens.

The cellular source of the cross-reacting antigens in placental tissue is unclear. By immunofluorescence, these antigens are widely distributed in the tissue. However, they do not appear on tissue-cultured cells of human origin. Moreover, the cell type that apparently possesses the greatest amount of Ad-2-related RNA as determined from *in situ* hybridisation, and thus is presumably most likely to synthesise most antigen, is relatively rare. One possible explanation is that different RNA molecules, related to different regions of the Ad-2 genome, are not equally abundant in all cells. Another is that the antigens or their precursors circulate in blood or other body fluids and/or that their distribution is altered as a result of post-mortem changes, such as blood clotting. Such a mechanism would partly explain why these antigens have not generally been found on cells in culture.

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letters

A jet associated with M89

AN optical jet, extending 10 arc min from the elliptical galaxy M89 (NGC4552) is reported here. Two low surface brightness shells partly surround the galaxy and a much fainter arc, 13 arc min away has also been detected. The jet was found on five deep plates of the Virgo cluster obtained by the 1.2-m UK Schmidt Telescope. Three sky-limited IIIaJ (bandpass 395–530 nm) and two IIIaF (630–685 nm) plates were taken on hypersensitised[1] emulsions. A slightly different plate centre was used for two of the plates.

The faintest objects on the plates were made visible by a contact copying process designed to detect very low surface brightness structures[2]. High contrast positives from the deepest IIIaJ plates were superimposed in register to reduce grain noise (Fig. 1). Low contrast detail, buried in the envelope of M89 was revealed by using an unsharp mask[3] to remove the large-scale density variations in the images. Objects smaller than ~1 arc min were unaffected by the mask and were further emphasised by the contrast enhancement process. Three of these high-contrast spatially-filtered positives were again superimposed in register. All the features clearly discernable on either kind of derivative made from at least two of the original plates (before superimposition) are sketched in Fig. 2, which is reproduced to the same scale as Fig. 1. A similar series of processes, using the same set of plates, was carried out on the superficially similar galaxy M84 (NGC4374). No unusual features were found.

All the derivatives from each of the plates clearly show a jet (A in Fig. 2) at a position angle of 115° extending 10 arc min (~36 kpc) from the nucleus of M89. The jet is brighter towards its tip but retains the same apparent width (~5 kpc) until it merges with the outer envelope of the main galaxy. The jet itself is slightly curved and contains some brighter condensations. One of these, (E) is off the axis of the jet and could be a background object. The outer envelope of the galaxy shows a distinct bulge on the eastern side, opposite the jet. To the south a sharply defined arc (B), reminiscent of a supernova remnant

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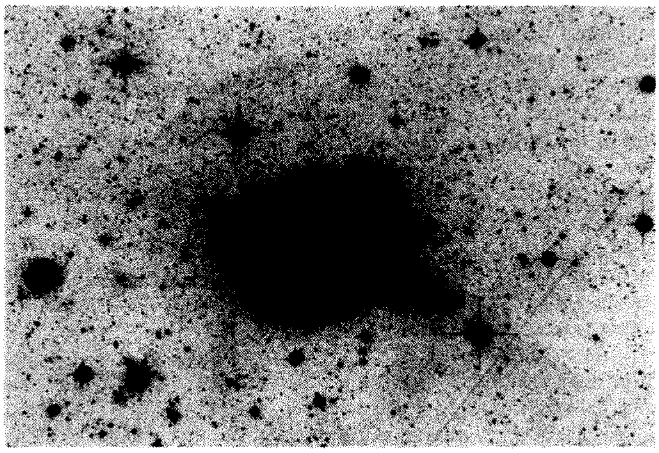

Fig. 1 Superimposed high contrast copies of two sky-limited IIIaJ plates showing the jet and three concentric shells. Same scale as Fig. 2.

shell, is visible, concentric with the nucleus of M89 and ~11 kpc long. The jet and southern arc are more obvious on the blue plates than on the red and are just visible on the originals. Diametrically opposite the southern bright rim and at a similar distance from the nucleus is a more diffuse arc (C) which appears as a circular patch on the red plates. The condensation D and extremely faint arc F, 47 kpc from the nucleus are evident only on the two deepest IIIaJ plates.

The dotted circle in Fig. 2 is the equivalent apparent radius of M89 at a (B) magnitude of 26.91 arc s^{-2} obtained from the photographic photometry of Fraser[4]. The circle is centred on the position of the optical nucleus.

In view of their low surface brightness, the nature of the jet and shells will be difficult to establish. Although the jet is of comparable brightness on the blue and red plates, suggesting that it is continuum emission, it could have a spectrum exhibiting [O III] 4959 and 5007 and Hα emission lines. This would be hard to reconcile with the known lack of interstellar gas in normal elliptical galaxies. Pure continuum could indicate synchrotron radiation, but no radio jet was found in observations by Heeschen[5]. The jet is therefore probably composed of stars. Tidal interaction with another galaxy cannot be discounted as the disruptive force but there seems to be no likely candidate in the vicinity and that mechanism would not account for the active radio nucleus or the concentric shells. The nucleus is a compact radio source, with a flat spectrum similar to that found in variable QSOs. The nucleus of M89 is variable at 6 cm (R. D. Ekers, unpublished data). The optical spectrum is unique among the limited number of active radio galaxies examined by Disney and Cromwell[6] in that it exhibits no emission lines. It seems likely that the jet and concentric shells are stars displaced by one or more violent outbursts in the active nucleus of the galaxy.

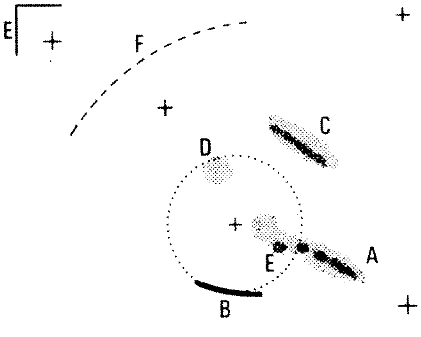

Fig. 2 Location of low contrast features visible in the spatial frequency-filtered derivatives of three IIIaJ plates.

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Theory of the Earth-synchronous rotation of Venus

CARPENTER[1] noted in 1966 that the radar observations of the rotation period of Venus gave a figure remarkably close to one at which Venus would present the same aspect to the Earth at each inferior conjunction. Goldreich and Peale[2] discussed this as a possible resonance with a gravitational couple exerted by the Earth on a permanent transverse quadrupole moment of Venus, but noted that such a couple would be expected to be much smaller than that due to the Sun acting on the solid body tide. The rotation would continue to slow down due to tidal friction, and could not have been arrested in the resonance. We therefore proposed[3] that the solar couple on thermal atmospheric tides might be expected to apply an acceleration to the spin of Venus, thus opposing the body tidal frictional deceleration. (An effect in that sense occurs for the Earth.) The two opposed torques would have a different dependence on the rotation speed of Venus, and might balance at a particular value. As the rotation speed approached this value, the torque necessary for capture into a resonance would have become very small, and the effect exerted by the Earth might then be sufficient. A diurnal pressure wave of as little as 1 mbar could balance the body tide. Ingersoll and Dobrovolskis[4] have reconsidered this suggestion, and attempted a quantitative calculation of the atmospheric tide. Although their result seems to favour the theory, we show here that their approach is not valid. Ingersoll and Dobrovolskis consider a quasi-static atmosphere heated diurnally, and then calculate the mass distribution and hence the solar couple that results. But the quasi-static assumption is not justified for the known circumstances on Venus. Wind speeds must be expected[5], and have indeed been observed[6], that cause an advection of heat substantially faster than the motion of the subsolar point over the surface (~4 m s^{-1}); in these circumstances the atmospheric mass distribution will be determined by the dynamics of the convecting atmosphere. (The acceleration terms rather than temperature make the main contribution to horizontal variations of pressure and density.) A secure result cannot be obtained until the flow patterns and velocities are known, from a detailed theory of planetary convection, or from direct observation.

Some considerations can immediately be used to show that the dynamical effects are likely to be as strong or stronger than we showed to be needed for the resonance capture. First, wind speeds on Venus are fast relative to the planet's rotation rate, unlike the case for the Earth. The slow diurnal rotation (117 d) and the deep, radiation-absorbing atmosphere of Venus make it probable that most of the global convection pattern is relatively stationary with respect to the subsolar point, rather than being carried around with the planetary rotation as is the case for the Earth. Cloud pattern photography, for example, of the permanent disturbance downwind from the subsolar point, substantiates this, but is available as yet only for the upper atmosphere[7]. The atmospheric mass asymmetries created by this motion are subject to a continuous solar torque. Indeed they may help produce the high altitude 4-d circulation[8].

Second, the wind speeds of such an advection have been estimated by several authors[9], and speeds of the order of 5 m s^{-1} are expected in the densest levels of the atmosphere (above the surface boundary layer). On a slowly rotating planet, the dynamical pressure fluctuations associated with this, such as the pressure drop in a large eddy, are then of the order of ρv^2, where ρ is the density and v the wind speed. This suggests that variations of 15 mbar in the surface pressure are to be expected, a figure substantially larger than the minimum of 1 mbar that we showed to be adequate for the tidal stability of the resonant rotation.

Soviet measurements[6] with Veneras 9 and 10 found wind speeds of 40–50 m s^{-1} down to 30 km above the surface. The corresponding value for ρv^2 is ~180 mbar, and similar absolute values of the pressure fluctuations are then expected to occur down to the surface. These measurements, which generally confirm the calculations of Gierasch *et al.*[9], also indicated that wind speeds were similar over a substantial (~20°) range of longitudes[10], making it clear that they were associated with large-scale patterns rather than merely small-scale turbulence that would be inconsequential for tidal effects. The figure of 180 mbar is so much in excess of what is required that the distribution of the atmospheric mass assymetry can be very inefficiently organised and still be enough to stabilise the resonant rotation against solid body tidal drag.

We conclude that the dynamical effects of convection will give the Venus atmosphere asymmetries in its mass distribution with respect to the Sun that are fully expected to provide an adequate couple for the Earth-resonance theory. A more detailed discussion depends on an improved understanding of the entire atmospheric dynamics.

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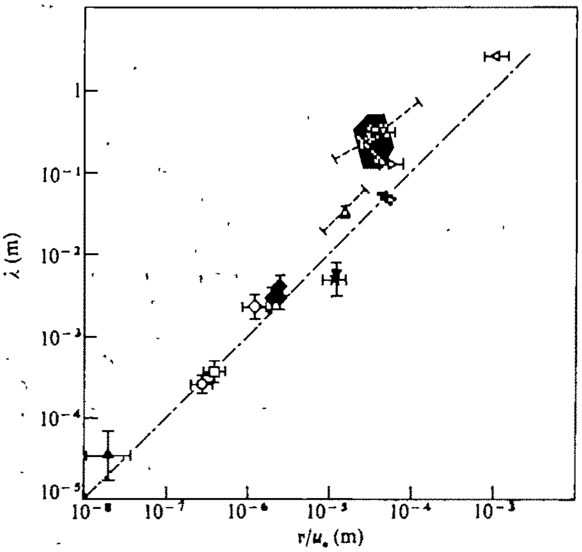
Size of scallops and ripples formed by flowing water

THE passage of water over a soluble solid wall often gives rise to intersecting polygonal depressions or horseshoe-shaped pits known as scallops and flutes[1,2]. The geometry of these surface deformations is remarkably reproducible in given flow conditions, but there is no generally agreed theoretical framework for predicting their characteristic dimensions, such as the spacing between successive transverse ridges. I present here indirect evidence in support of the hypothesis that the scalloping of soluble surfaces is due to the imprint of eddy patterns inherent in turbulent flow near a passive wall, and argue that small ripples on a granular bed may be formed in essentially the same way.

The attribution of bedform morphology to the structure of wall turbulence, rather than to the physical properties of the bed itself, has been suggested several times[3–6]. We may call this basic assumption the wall-similarity hypothesis, since it implies by dimensional arguments referred to in the turbulence literature as the principle of wall similarity[7], that a characteristic length scale of the bedform should be a universal multiple of ν/u_*, where ν is the kinematic viscosity and u_* is the friction velocity. Although the wall-similarity hypothesis has been shown to account successfully for the size of scallops formed by dissolution both in geophysical flows[5] and in laboratory studies[6], its validity range is uncertain[1], and in particular no attempt has been made to apply it to features observed on granular beds. Among the diverse bedforms associated with non-cohesive sand, those described as ripples[8] resemble dissolution scallops most closely in size, and hence have the strongest claim to an explanation in terms of the wall-similarity hypothesis. However, it has been demonstrated experimentally[9,10] that ripple wavelength does not depend in a simple linear manner on ν/u_*, and this has been taken to mean that wall-similarity concepts are not relevant to sand beds[11].

Most of the literature on bedform morphology refers to flows occurring in nature or to laboratory simulations of geophysical flows, but several instances have been reported of flow-induced wall deformations in man-made industrial plant. A striking example is the formation (probably by a dissolution process) of



key	fluid	wall	remarks	ref
■	cold & warm water	granular, non-cohesive	laboratory flume	9
•	"	"	"	own expts
○	hot water	magnetite	boiler tubes	12–18
△	cold & warm water	plaster	laboratory channel	6
▷	cold water	rock	natural formations	5
◁	air	ice		
▼	cold water	colloidal particles	deposits in a water main	24, 25
▽	"	ice	ripples on the underside of river ice	26
◆	warm water	bitumen	laboratory pipe-flow expts	22
□	cold & warm water	copper	laboratory studies of erosion corrosion in pipes	21
◇	cold water	brass	"	20
▲	water at 750 m s^{-1}	aluminium	laboratory studies of erosion by droplets	23
×	cold water	machined ripples	see text	37

Fig. 1 Streamwise spacing of flow-induced surface deformations, λ, plotted against the wall-similarity parameter, ν/u_*. Chain-dotted line: $\lambda = 10^3\,\nu/u_*$. Broken lines inclined to the axes indicate the range of conditions covered by the original references.

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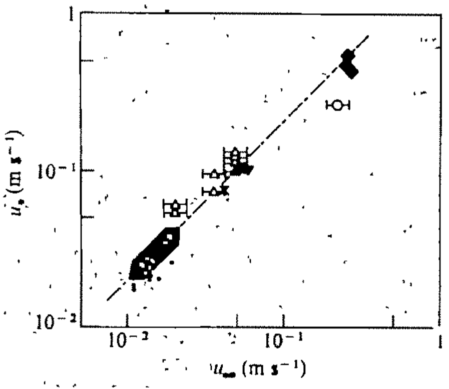

Fig. 2 Empirically determined friction velocity, u_*, for flow over naturally scalloped or rippled surfaces, plotted against the friction velocity, u_{*0}, calculated or measured for the same bulk flowrate over a smooth wall. For the key see Fig. 1. Chain-dotted line: $u_* = 2u_{*0}$.

rippled layers of magnetite on the internal surfaces of boiler tubes carrying hot water at a velocity of a few metres per second[12-18]. The morphology of the deposits is very like that of cave scallops or river-bed ripples, but the longitudinal wavelength is much smaller, of the order of 200–350 μm. Such a reduction of scale agrees well with wall-similarity predictions.

Several published accounts of wall deformations generated by flow are summarised in Fig. 1. The reported morphologies differ in detail, but all the deformations can be described as either scallops or ripples, displaying a definite transverse character and a well-defined average spacing, λ, in the direction of flow. Some of the original papers do not give sufficient information to permit exact calculation of ν/u_*. In these cases, plausible assumptions have been made regarding water temperature or Reynolds number, and where necessary u_* has been taken to be twice the friction velocity appropriate to a smooth wall at the same bulk flowrate, as calculated using the Moody chart[19] for pipe flows or by assuming a universal logarithmic velocity profile[7] for the flume and channel flows. This method of estimating u_* was based on an analysis of those sources giving information on the increase in resistance to flow accompanying the formation of scallops and ripples (Fig. 2).

The approximate proportionality apparent in Fig. 1 between λ and ν/u_* over a range exceeding four decades of length represents strong support for the wall-similarity hypothesis. Most of the data are fitted within a factor of two or three by the formula $\lambda = 10^3\,\nu/u_*$. Figure 1 includes some of my experimental points, obtained using a variety of granular beds, of order 1 mm thick, in a small laboratory flume. My results, considered in isolation, do not show the trend $\lambda \propto \nu/u_*$, but the group of points taken as a whole falls on the same line as data referring to the corrosive dissolution of steel[12-18], brass[20] and copper[21], the plastic shear of bitumen[22] and aluminium[23] and the rippling of colloidal-particle deposits in a water main[24,25]. On deeper sand beds[9], ripple wavelengths are larger by a factor of ~4 than those obtained in my tests, suggesting that the height attained by ripples, s, may influence their spacing; this idea is supported by the fact that as ripples develop from an initially plane bed, their wavelength increases until eventually stabilising as an equilibrium ripple height is reached[27]. Linear regression applied to the deep-bed data in the form $\lambda = A\nu/u_* + Bs$ yielded $A = 1{,}250$, in satisfactory agreement with my shallow-bed results, and $B = 13.3$, a value consistent with the commonplace visual observation that in the lee of a ripple crest there is an unsteady recirculation zone extending a distance of the order of $10s$ downstream. However, allowance for ripple height does not adequately account for the anomalously large spacing of river-ice ripples[26], and it is possible that additional corrections to the basic wall-similarity estimate, as yet unidentified, will prove necessary for the accurate prediction of λ.

As there is no systematic divergence in Fig. 1 between data points relating to the rippling of granular beds or plastically deformable surfaces and points referring to dissolution processes, it seems that the applicability of the wall-similarity hypothesis is not limited to dissolution scallops alone. The evidence suggests the size of both scallops and ripples is determined primarily by the scale of pre-existing eddies in the adjacent turbulent flow. If circumstances permit the surface deformations to become so pronounced that extensive regions of separated flow are set up behind crests, the morphology and characteristic dimensions of the deformations will in general be modified, but it seems that this should properly be regarded as a secondary complication. Flow separation may perhaps be best considered as responsible merely for furnishing final sculptural details, determining, for example, whether scallops are formed rather than ripples, while the overall scale of the bedform has its origin in eddy patterns already present in the flow before surface irregularities have become sufficiently marked to induce separation. (These remarks do not, of course, apply to large-scale bedforms such as dunes or antidunes.)

If this perspective is accepted, the fact that the wavelength of ripples on a granular bed does not vary directly with ν/u_* is no longer irreconcilable with wall-similarity ideas. Suppose, for example, that the amount by which u_* exceeds the threshold of movement is a factor affecting bedform length scale, possibly through its influence on ripple profile and hence on the extent of the separation zone in the lee of a crest. Such a second-order effect, although subsidiary in the sense that it operates in the context of a bedform whose characteristic dimensions are fixed principally by turbulence length scales may, nevertheless, mask the simple linear dependence of λ on ν/u_* when the latter quantity is varied over the necessarily restricted range available in flume experiments, where u_* is bounded below by the threshold of movement and above by the necessity of preventing the ripples giving way to a different bedform regime.

The wall-similarity hypothesis for the rippling of granular beds may also provide an explanation for the absence of ripples when grains are larger than a critical size. For grain Reynolds numbers which are not too small, the Shields criterion is[8] $u_{*0} = 0.2(\Delta\rho g d/\rho)^{1/2}$ where u_{*0} is the friction velocity at the threshold of movement, ρ is the water density, $\Delta\rho$ is the difference in density between particle and fluid, d is the particle diameter and g is the gravitational acceleration. The maximum observable ripple wavelength for a bed of grain size d is therefore

$$\lambda_{max} = 1{,}000\,\nu/u_{*0} = 5{,}000\,\nu(\Delta\rho g d/\rho)^{-1/2}$$

But as the height of a fully developed ripple is usually no more than a tenth of its wavelength, a ripple will be indistinguishable from background bed roughness unless $\lambda_{max} > 20d$, say. Hence the grain size above which rippling would not be observed may be estimated as

$$d_{max} = (250\nu)^{2/3}(\Delta\rho g/\rho)^{-1/3}$$

Inserting physical properties appropriate to sand and water at ambient temperatures, the predicted d_{max} is 1.6 mm, which compares favourably with the value of about 0.7 mm found empirically[11].

Although no mechanistic explanation can be given for the value of the constant of proportionality between λ and ν/u_*, its order of magnitude suggests that the eddies responsible for the formation of scallops and ripples may be identified with the coherent flow structures known as sublayer bursts[28,29]. The dynamics of sublayer bursts are not understood, but empirical studies of turbulent flow over a smooth wall have established that they are elongated in the streamwise direction, with a typical lateral spacing[30-33] of 100 ν/u_* and a longitudinal length scale[29] in the approximate range 1,000–1,500 ν/u_*. Relatively few experiments have been performed to examine how the behaviour of sublayer bursts is modified by surface roughness[34,35], but in the case of a wall roughened by transverse ribs the streaky structures associated with bursts have been observed in the regions between ribs[34]. The bursts, which near a smooth surface occur randomly in position and time, possibly become locked into the irregularities of a deforming wall, thereby imposing bedform dimensions which reflect their own characteristic longitudinal scale, approximately $10^3\ \nu/u_*$. This speculation receives some support from evidence that bursts can impose their lateral length scale when longitudinal grooves are formed on a soluble[2] or erodible[35,36] wall. It is also interesting that as the flowrate is varied over a wall into which artificial ripples have been machined[37], the resistance to flow passes through a maximum when the ratio between ripple spacing and ν/u_* is $\sim 10^3$ (Fig. 1). Such a resonance phenomenon is consistent with the postulated tendency of sublayer bursts to lock into a spatially periodic pattern of transverse ridges: at resonance the surface morphology can be envisaged as giving the maximum possible assistance to the bursts in projecting low-momentum fluid towards the core of the flow, augmenting the Reynolds stress.

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Iron–nickel 50–50 superstructure in the Santa Catharina meteorite

MÖSSBAUER effect and X-ray diffraction measurements have shown that thin lamellae of the Cape York and Toluca meteorites contain the ordered phase Fe–Ni (50–50%) with the structure L10 (refs 1–3). The bulk of these meteorites consists mainly of body centred cubic (b.c.c.) iron–nickel α-phase alloy. We report here Mössbauer spectra, electron microprobes and X-ray measurements which show that in contrast to this, the bulk of the Ni-rich ($\sim$35%) Santa Catharina Ataxite consists of up to 50% Fe–Ni superstructure[4].

Thin slices of the bulk of the Santa Catharina meteorite from specimens of the collection from the Museu Nacional in Rio de Janeiro were cut with a precision saw, carefully avoiding thermal stresses in the sample. Absorbers for Mössbauer spectra measurements were prepared by polishing the slices to a thickness around 70 μm. The spectra were recorded with a 20 mC source ^{57}Co in Rh matrix with a velocity drive coupled to a multichannel analyser of 1,024 channels. A 370/145 IBM computer was used for folding and analysing the data with a lorentzian least square fitting.

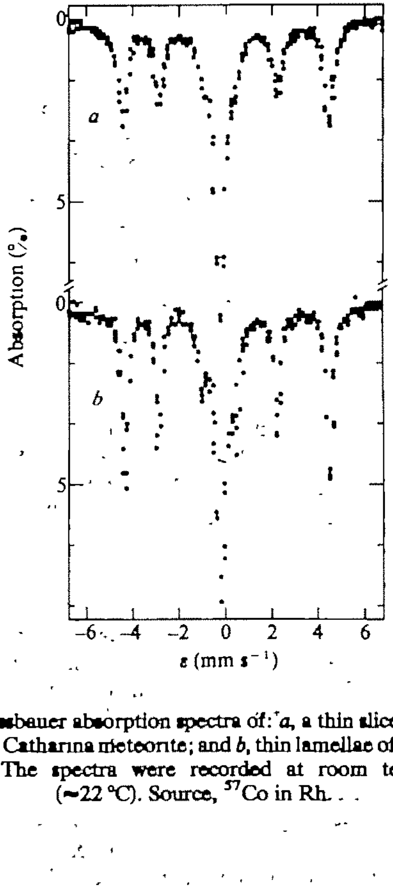

Fig. 1 Mössbauer absorption spectra of: *a*, a thin slice ($\sim$70 μm) of the Santa Catharina meteorite; and *b*, thin lamellae of the Toluca meteorite. The spectra were recorded at room temperature ($\sim$22 °C). Source, ^{57}Co in Rh.

Figure 1 compares a spectrum of the Santa Catharina sample with one obtained in the same conditions with a thin lamella of the Toluca meteorite, in which X-ray determinations showed the presence of the superstructure[3]. Both spectra are a superposition of two spectra: (1) a central line from a paramagnetic phase; and (2) an asymmetric 6-line spectrum, due to the L10 superstructure, which gives rise to a quadrupole splitting caused by the non-cubic surroundings of the iron atoms in this alloy[5–7]. The magnetic hyperfine field H_i and the electric quadrupole shift ε of the Santa Catharina sample are about the same as those observed with the lamella (lamella: $H_i = 289 \pm 2$ kG, $\varepsilon = 0.20 \pm 0.02$ mm s^{-1}; Santa Catharina: $H_i = 291 \pm 3$ kG, $\varepsilon = 0.17 \pm 0.02$ mm s^{-1}). The linewidths obtained with the Santa Catharina ($\Gamma \sim 0.52$ mm s^{-1}) are larger than those obtained with the Toluca lamella ($\Gamma \sim 0.32$ mm s^{-1}). This may be due to the larger thickness of the sample. Only minor changes, both in the paramagnetic central line and in the magnetically split Mössbauer spectrum, were observed on going from room temperature to liquid nitrogen (77 K) and liquid helium (4.2 K) temperatures. This surprising result, will be discussed in detail elsewhere.

As has been observed with the Cape York lamellae[2], the Mössbauer spectra of samples from the Santa Catharina meteorite at 732 K show that the breakdown of the superstructure occurs over a time scale of $\sim$10 h.

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From our results, the area ratio of the 6-line spectrum to the single line, we conclude that ~50% of the Santa Catharina meteorite consists of the ordered Fe–Ni alloy with L10 structure. In the limits of sensitivity of the Mössbauer effect no evidence was found for other iron-containing minerals.

Electron microprobe (Microscan 5, Cambridge Scientific Instruments) analysis of the polished Santa Catharina samples indicates clearly the presence of at least two phases[8] as shown in Fig. 2. Microanalysis at points of the two phases indicate, from an average of 50 determinations, that the darker phase has a homogeneous composition, 51–50% (by weight) of Ni and 49–48% of Fe and that the lighter phase has a more heterogeneous composition containing nearly 27% of Ni. The meteorite consists of an intergrowth of orientated domains of these two phases, which implies that slices cut from the meteorite will always contain both phases. The presence of oxygen and sulphur in maximum amounts of 1–2% were found uniquely on the Ni-rich phase. Larger proportions of oxygen have been reported in this phase from earlier microprobe work[9]. The discrepancy may be due to the fact that earlier measurements were made on a severely oxidised sample. The fact that our samples taken from the bulk of the Santa Catharina meteorite show only traces of corrosion demonstrates that care has to be taken when results from different specimens of the Santa Catharina meteorite are compared[10]. We have also studied the sample from Santa Catharina by X-ray techniques. Oscillating crystal photographs show that the sample over areas of at least 1 mm^2 is essentially a single crystal, and that it contains the superstructure L10 of Fe–Ni. The X-ray photographs showed no traces of martensitic α_2 phase.

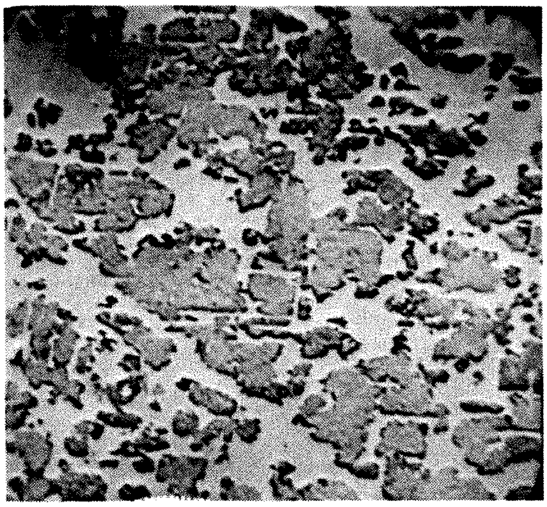

Fig. 2 Back-scatter electron image from electron microprobe analysis of a polished surface of the Santa Catharina sample. From point analysis determination it is found that the dark areas correspond to the Fe–Ni ordered phase whereas the light ones correspond to the Fe-rich paramagnetic phase.

Remarkably ~50% of the Santa Catharina meteorite, which originally may have been a 25,000 kg meteorite[11], consists of the ordered Fe–Ni structure. This structure has only been obtained by strong neutron or electron irradiation of disordered alloys at temperatures near the ordering temperature[5–7] ($T_c \approx 320$ °C). Diffusion processes substantially below T_c are too slow to obtain thermodynamic equilibrium[12]. The presence of the ordered Fe–Ni in Santa Catharina and other meteorites[1–3] is probably due to the slow cooling of the meteorite parent body.

Further experiments are in progress to clarify the nature of the iron-rich paramagnetic phase as well as the magnetic properties of the ordered Fe–Ni phase.

Microprobe analysis were performed by H. Schorscher, C. Wiedmann, and C. Jehanno.

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Paraffinic hydrocarbons from supercritical-gas extracts of coal as organic geochemical markers

PARAFFINIC hydrocarbons have been proposed as indicators of the conditions obtaining during the deposition and subsequent maturation of sediments[1–4]. We consider here the relationship between the rank (as measured by carbon content) of coals and analytical results for the alkane fractions obtained by a novel extraction procedure (under investigation for the derivation of liquid fuels and feedstocks from coal[5,6]) which uses super-critical gases (SCG). We first discuss the limitations of *n*-alkane distributions and then deal with acyclic isoprenoids, drawing particular attention to the ratio of pristane to phytane.

In the *n*-alkane fractions of coals, like those from some other sediments, odd carbon numbers predominate over even[3]. For a series of Saar coals the carbon-preference index (CPI) varied systematically from 1.59 to 1.00 with degree of coalification[7], and a related parameter dropped[8] from 15.3 to ~1.0 for Australian coals as the carbon content changed from 67% to over 90%. On the other hand, the CPI of *n*-alkanes from hatchettites (coal-associated minerals) varied only between 0.96 and 1.06 (ref. 9). Recently, White *et al.* have questioned the correlation between *n*-alkane distributions and coal rank for paraffins from coal hydrogenated in the SYNTHOIL process[10].

We have isolated paraffinic hydrocarbons from a variety of coal derivatives by silica-gel chromatography of the portion soluble in *n*-pentane. Gas chromatographic analyses were then made on both packed and capillary columns for whole alkane fractions and for the straight-chain and branched-plus-cyclic alkane fractions separated on a molecular sieve[11–13]; mass spectrometry enabled unambiguous identification of individual alkanes[12]. Table 1 and Fig. 1 present our results for the distributions of *n*-alkanes derived from SCG and Soxhlet extraction, and low-temperature carbonisation from UK, Turkish and

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Table 1 Origin of coal extracts and properties of derived aliphatic hydrocarbon fractions

Coal source	% Carbon of coal (dry ash-free basis	Origin of extract	Aliphatic fraction % of coal	*n*-Alkanes % of coal§	CPI* of *n*-alkanes	Significant† concentration of alkenes	Isoprenoids p.p.m. of coal§	Pr/Ph	Ref.
Turkish lignite 1 (Elbistan)	67.3	SCG extraction at 340 °C and 8 MPa‡	0.7	0.45	1.47	No	150	1.6	This work
		SCG extraction at 400 °C and 17 MPa	2.0	0.8	1.16	Yes	ND	ND	This work
Turkish lignite 2 (Seyitomer)	71.1	SCG extraction at 340 °C and 8 MPa	0.7	0.5	1.13	No	120	2.0	This work
Turkish lignite (Tunçbilek)	74.6	SCG extraction at 400 °C and 17 MPa	ND	ND	0.99	Yes	ND	1.0	This work
Sigma, South Africa	76.7	SCG extraction at 350 °C and 17 MPa	0.09	0.06	1.33		NA	NA	14
		SCG extraction at 450 °C and 17 MPa	0.5	0.3	1.01	Not stated	NA	NA	14
		Soxhlet extraction with 3:1 chloroform/methanol for 100 h	0.05	0.03	1.93		NA	NA	14
Pittsburgh, US	80.6	Low-temperature carbonisation (COED process)[25]	NA	NA	1.02	Yes	NA	6.0	This work
Thoresby, UK (CRC 801)	81.4	Low-temperature carbonisation (Rexco process)[26]	~0.5¶	~0.3¶	1.02	Yes	~200¶	4.3	This work
Daw Mill, UK (CRC 902)	82.6	SCG extraction at 400 °C and 10 MPa	0.6	0.3	1.04	Yes	200	2.9	This work
		Soxhlet extraction with 7:3 benzene/ethanol for 50 h	0.07	0.03	1.07	No	70	2.6	This work
Markham Main, UK (CRC 802)	82.7	SCG extraction at 350 °C and 10 MPa	0.35	0.2	1.09	No	500	5.6	11
		SCG extraction at 400 °C and 10 MPa in presence of $H_2/ZnCl_2$	1.6	0.5	1.08	No	500	3.5	This work
		Low-temperature carbonisation at 430 °C (Roomheat process)	~0.4¶	~0.3¶	1.02	Yes	~150¶	4.4	This work and 15
		Soxhlet extraction with 7·3 benzene/ethanol for 50 h	0.08	0.04	1.27	No	110	4.2	This work

NA, not available; ND, not determined.

* CPI = $\frac{1}{2}$\{odd (C_{15}–C_{29})/even (C_{14}–C_{28}) + odd (C_{15}–C_{29})/even (C_{16}–C_{30})\}

† >0.01% of extract regarded as significant

‡ Toluene, used as solvent for all SCG extractions, yielded insufficient Soxhlet extract for alkane analysis.

§ GC conditions. 50 m × 0.3 mm glass capillary column coated with OV-101 programmed from 373 to 473 K at 1.5 K min^{-1}, and for higher paraffins, a 2 m OV-1 column.

¶ Based on 10% tar yield[26].

US coals, together with Kershaw's data for extracts of a South African coal[14]. By volatilising liquids otherwise insufficiently volatile to distil at the temperatures used, the SCG extraction process[5,6] yields much greater amounts of paraffins than conventional extraction procedures, but without appreciable hydrogen transfer[11].

The low C_{10}–C_{15} *n*-alkane contents in Fig. 1*a*, *c*, *d*, and *g* provide some evidence that the alkane fractions are not significantly degraded during SCG extraction over brief periods at around 340 °C. Indeed, although SCG extraction at 350 °C of the Markham Main (UK) and Sigma (South African) coals produces much greater quantities of *n*-alkanes than the mild Soxhlet extraction process (Table 1), the *n*-alkane range is almost identical (compare Fig. 1*d* with *f*, and *g* with *i*). The virtual absence (<0.01%) of alkenes in low-temperature SCG extracts (Table 1) also suggests a very low degree of pyrolysis. On the other hand, alkanes from a tar generated by fluidised-bed carbonisation[15] of Markham Main coal showed a roughly gaussian distribution (Fig. 1*h*), with a shift to lower carbon number compared with SCG extraction (Fig. 1*g*); significant quantities of alkenes detected in this and other low-temperature tars (Table 1) are thought to arise by de-methanation and de-ethanation of *n*-alkanes. Moreover, even SCG extraction at only 400 °C generated alkenes from Turkish lignites, and shifted the *n*-alkane distribution from that of a 340 °C extract (compare 1*a* with 1*b*); the CPI fell from 1.47 to 1.16 (Table 1). Alkenes were also apparently present in the 400 °C SCG extract of Daw Mill coal[13]. CPI values for the *n*-alkanes derived from Kershaw's data[14] also illustrate the effect of increasing the SCG extraction temperature from 350 °C (CPI 1.33) to 450 °C (CPI 1.01). Other SCG extracts at 400 °C, as well as low-temperature tars, exhibit CPI values near unity (Table 1).

Because it yields much larger quantities (by up to two orders of magnitude) of paraffins, SCG extraction without pronounced cracking, should provide alkanes that are more representative of the whole coal than does simple extraction (which yields only 0.007–0.099%, in refs 8, 10 and 14 and this work). Now, two sources of alkanes in coals must be considered: (1) the material physically absorbed in the micropore structure[16]; and (2) the compounds generated by chemical breakdown of oxygen-containing compounds, or long alkyl chains, shown[13] by ^{13}C NMR to be bonded within the coal matrix.

Vahrman attributed the increasingly smooth carbon-number distribution of *n*-alkanes from coal as extraction times become more extended to the differences in accessibility of the *n*-alkanes with high CPI (from the leaves, cuticle, and so on of the plants from which the coal is derived) as compared with those of lower CPI (originating from heartwood)[16]. A similar effect is evident when one compares CPI 1.27 for Soxhlet extraction (benzene–ethanol gave better yields than toluene) with CPI 1.09 for SCG extraction at 350 °C of the Markham Main (UK) coal; for the South African coal, the CPI decreases from 1.93 to 1.33 for analogous extracts. However, Allan *et al.* have shown that moderate temperature increases (from 275 to 375 °C for 24 h) can substantially change *n*-alkane distributions from UK vitrinites[17]. Evidently the extra alkanes generated on heating vitrinites have a different degree of odd/even predominance from those accessible to Soxhlet extraction; thus one cannot distinguish between the two processes, (1) and (2) above, as to the source of the extra *n*-alkanes in the SCG extracts. On the other hand, coal hydrogenation procedures, such as the SYNTHOIL process[10] and hydrogen-assisted SCG extraction (Table 1), clearly yield even larger quantities of *n*-alkanes, probably by hydro-cracking of aliphatic side chains.

Thus, apparent correlations between CPI and coal rank are almost certainly spurious because they are based on atypical proportions of *n*-alkanes obtained by simple extraction; furthermore, CPI values for *n*-alkanes derived by processes involving heating above 350 °C, or by hydrogenation, are also unreliable. SCG extraction at 340–350 °C may yield more representative samples, but the variety of origins of the alkanes extracted in this way renders the CPI of doubtful value in correlations with coal rank: CPI values for four coals from diverse sources show only a very approximate correlation with dry-ash-free carbon content (Table 1). All that can be said is that the large concentrations of C_{23} and higher *n*-alkanes are consistent with the predominantly land-derived nature of each sediment; a marine origin is typified by lower chain lengths[1,2] (Fig. 1).

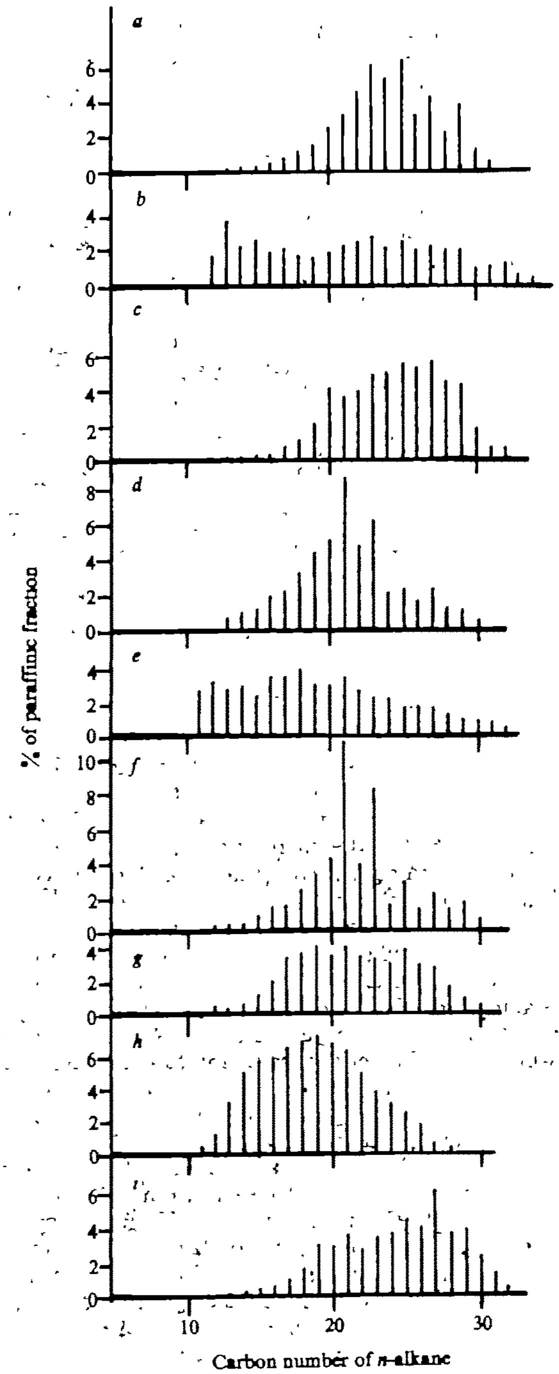

Fig. 1 Distributions of *n*-alkanes in paraffinic fractions of coals: *a*, Turkish lignite 1 (Elbistan), SCG extraction[27] at 340 °C; *b*, Turkish lignite 1, SCG extraction at 400 °C; *c*, Turkish lignite 2 (Seyitomer), SCG extraction[27] at 340 °C; *d*, Sigma (South African) coal, SCG extraction at 350 °C; *e*, Sigma (South African) coal, SCG extraction at 450 °C; *f*, Sigma (South African) coal, Soxhlet extraction with 3:1 chloroform/methanol for 100 h; *g*, Markham Main (UK) coal, SCG extraction at 350 °C; *h*, Markham Main (UK) coal, fluidised-bed carbonisation at 430 °C; *i*, Markham Main (UK) coal, Soxhlet extraction with 7:3 benzene/ethanol for 50 h. (*d*), (*e*) and (*f*) from ref. 14.

Acyclic isoprenoids may provide clearer evidence than *n*-alkanes of the geochemical history of sediments[2] as their major precursor is thought to be the phytyl side chain of the chlorophyll pigment from the original plant material. The presence of six derived hydrocarbons (C_{14}, C_{15}, C_{16}, C_{18}, C_{19} and C_{20} isoprenoids) has been demonstrated in numerous coal extracts and tars[3]. In Australian sub-bituminous coals[18], the pristane (C_{19}) content of the alkane fraction of (low-percentage) chloroform–methanol extracts begins to increase as carbon contents exceed 76%, but the phytane (C_{20}) concentration does not increase significantly until much later in the diagenetic process, corresponding to 83–85% carbon. At this stage the pristane/phytane ratio (Pr/Ph) is a maximum[18]. Parallel relations have been demonstrated between Pr/Ph and reflectance of New Zealand vitrinites[19], and with the maturity of shales from the Douala Basin[20]. The inter-relation of coal maturation and oil formation[18,21] is emphasised by correspondence between Pr/Ph for oils and the rank of coals in associated beds[22]. These changes during maturation, however, may not entirely erase the partial record of the palaeoenvironmental conditions during sedimentation provided by Pr/Ph[2]; by increasing the relative proportion of pristane to phytane, thermal diagenesis preserves Pr/Ph ratios greater than one, typical of deposition in oxic conditions[2,22].

In contrast with the CPI of *n*-alkanes, and in spite of the threefold increase in the quantity of isoprenoids liberated during SCG extraction, Pr/Ph ratios are similar for SCG and Soxhlet extracts and, even for low-temperature tars, from the same coal (Table 1). Moreover, even though the coals are of diverse origin, the Pr/Ph ratios still fit fairly closely to the curve for simple extracts determined for Australian coals by Brooks *et al.*[18]. The consistency of the Pr/Ph ratio in differing extraction conditions, and the similarity in thermal stabilities of isoprenoids reflected in the agreement for low-temperature tars, clearly make this parameter useful in discussing the geochemical history of coals, although more than one origin is possible for these compounds.

The isoprenoids form a higher proportion of the total paraffins from Soxhlet extracts than from SCG extracts, in keeping with Vahrman's suggestion[16] that branched alkanes are more accessible within the micropore structure. However, as for *n*-alkanes, the release of isoprenoids bonded within the coal structure must also be taken into account; Connan has shown[19] how larger quantities of pristane and phytane were extractable from lignite after heating for 3 days at 300 °C.

The completeness of extraction of isoprenoids by the SCG method is indicated by the similarity of the total contents (expressed as a fraction of the original coal) in products obtained at 350 °C and in the presence of hydrogen at 400 °C (Table 1). Indeed, for the Markham Main coal, isoprenoid content (with pristane predominant at as much as 300 p.p.m.) suggests that much of the chlorophyll-derived phytol remains in 'fossil' form. The biochemistry of Palaeozoic flora is obscure; modern descendants of such plants may differ markedly in their environments and occupy different ecological niches[23]. However, if a chlorophyll content[24] of 0.7–1.3% (dry basis) for higher plants including ferns and mosses is taken as appropriate, the total available phytol would then amount to between 2,000 and 4,000 p.p.m. of this, compared with 500 p.p.m. derived isoprenoids in the coal.

In conclusion, SCG extraction at about 350 °C causes little thermolysis and is favourable for extracting relatively large quantities of paraffins representative of the original coals. Because of the different nature of *n*-alkanes physically absorbed in the coal, and at least partly accessible to Soxhlet extraction, as compared with those chemically released by heating, the CPI is inappropriate for monitoring deposition and maturation. The isoprenoidal hydrocarbons, and especially the Pr/Ph ratio, are more widely applicable as geochemical markers, and valuable for commercial products derived from coal by newer liquefaction processes.

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Steroids ketones in surface sediments from the south-west African shelf

STEROIDS are a class of biologically produced compounds which may serve as tracers for the transformation processes of labile organic matter in sediments. Diagenetic alteration of steroid alcohols (sterols) by geochemical and biological processes often leads to the accumulation of transformed products in the sediments. By elucidating these structures and determining their concentrations we should be able to determine their reaction mechanisms and rates of degradation. We have previously postulated that steroid ketones (stanones) are intermediates in the diagenesis of sterols to sterenes, a group of unsaturated steroids detected in some marine sediments[1] (Fig. 1). We report here the isolation, identification, and geochemical significance of a series of 4-methyl stanones and their 4-desmethyl counterparts from anoxic surface sediments in an upwelling zone on the south-west Africanshelf, Walvis Bay, RSA. To our knowledge, this is the first report of stanones being observed in Recent marine sediments, although 4α-methyl stanones have been reported previously as major components of the immature Messel oil shale[2].

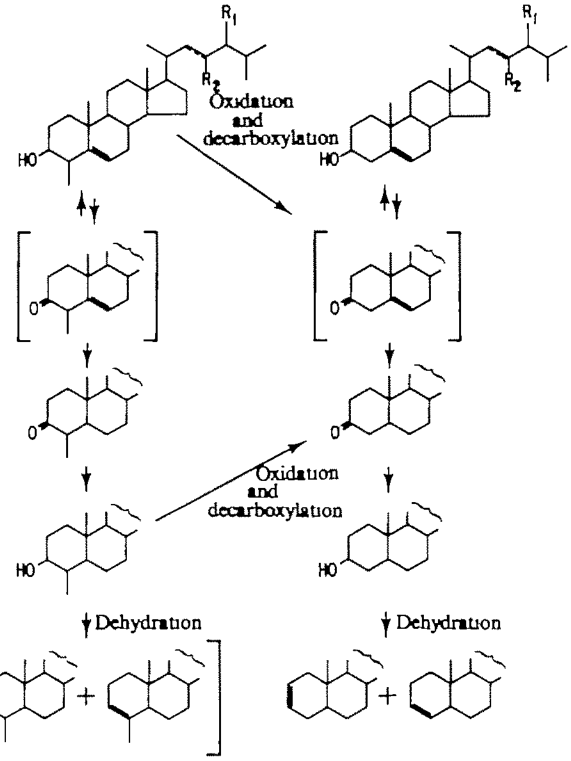

Fig. 1 A proposed mechanistic pathway for the microbiological or chemically mediated degradation in sediments of stenols to sterenes through stanone intermediates. Compounds in parenthesis are intermediates not isolated in this study.

Sediment samples were collected on RV Atlantis II Cruise 93, Leg 3, Capetown to Capetown, RSA in December 1975–January 1976. Core 16, taken from Station 11 at a water depth of 49 m at 22°47.5′ S, 14°25.8′ E, was analysed for its sterol (stenol + stanol), stanone, and sterene contents. The geology of this area has been described by Bremner[4,5] and the advantages of doing organic geochemical research in Walvis Bay by Boon *et al.*[6,7] and Morris and Calvert[8]. The sediment analysed was an organic-rich diatomaceous mud, composed mainly of frustules of *Coscinodiscus*, *Actinocyclus*, and *Thalassionema*. The top 4 cm of sediment were treated with 3 M HCl to remove the large amount of carbonate and Soxhlet extracted with benzene–methanol as previously described[3]. Column chromatography of this extract was performed on a 5% deactivated silica gel column using increasing amounts of ethyl acetate in hexane as eluant[3]. Fraction 1, eluted with hexane, contained various alkanes, cycloakanes, alkenes, cycloalkenes, and sterenes. Fraction 6, eluted with 15% ethyl acetate in hexane, contained the steroid ketones. Androstane was added as an internal standard. Blanks were also analysed during the extraction, isolation and analysis and contained negligible concentrations of stanones in the samples. 5α-Cholestan-3-one was added to pre-extracted sediment in the extraction thimble and used to quantify stanone recoveries. Mass spectra were obtained using a Varian aerograph 1400 gas chromatograph equipped with an SE-52 glass capillary column (25 m × 0.30 mm i.d.) interfaced to a Finnigan 1015 C quadrupole mass spectrometer[1]. The mass spectrometer

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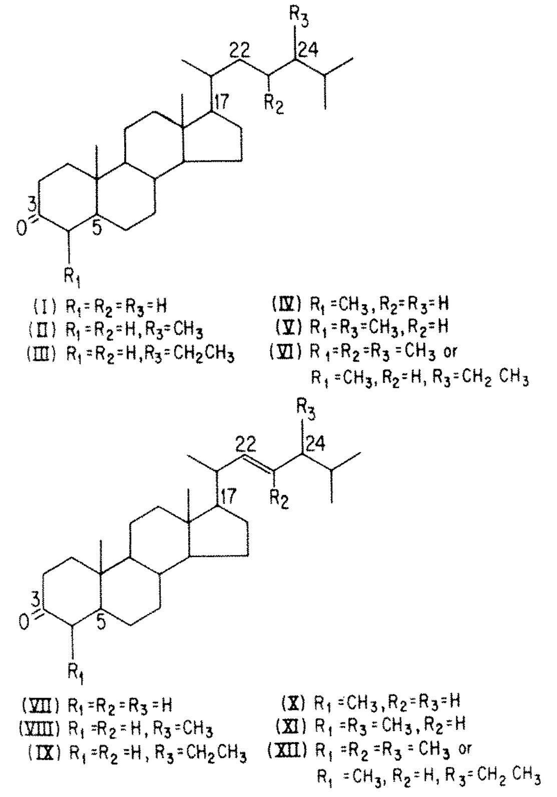

Fig. 2 Molecular structures of stanones isolated from surface sediments from 22°47.5′ S, 14°25.8′ E.

is interfaced with a Systems Industries 150 data system with DEC PDP 8-E (8K core) computer. The carrier gas (He) flow rate was 2–3 ml min^{-1}, the injector temperature was 250 °C, and the column was programmed from 70 to 260 °C at a rate of 4 °C min^{-1}. A more detailed description of the analytical methodology will be published elsewhere.

Four series of stanones were identified (Fig. 2) which differed primarily in (1) unsaturation at the C-22 position; (2) methylation at C-4; and (3) alkylation at C-24. 4,23,24-Trimethylcholest-22-en-3-one or 4-methyl-24-ethylcholest-22-en-3-one (XII) and 4,23,24-trimethylcholestan-3-one or 4-methyl-24-ethylcholestan-3-one (VI) were the major components, found in concentrations of 2.5 μg per g and 1.0 μg per g dry weight sediment, respectively. These structural identifications are based on published mass spectra and fragmentation patterns of other stanones[9–12]. However, as the mass spectrum of the 4,23,24-trimethyl compound is essentially the same as that of its 4-methyl-24-ethyl isomer, a definitive structure proof will have to await further separation and NMR studies[9]. Other stanones were detected at concentrations ranging from 3 to 16% of (XII), with the exception of (IV) and (X) which were found in concentrations of <1%. Structural elucidation of the stanones was based on relative retention times on high resolution glass capillary gas chromatography and comparison of GC–MS spectra with published mass spectra[11–17] or mass spectra obtained from authentic standards (I and IX). Mass spectral fragmentation of the Δ22 unsaturated stanones was dominated by ions corresponding to cleavage of the side chain, bonds C-17–C-20 and C-20–C-22 (Fig. 3*a*)[10,17]. The major fragments of the saturated stanones arose from cleavage of ring D (Fig. 3*b*)[11]. In addition, both the 5α and 5β isomers of (I) and (II) were detected, the 5β having an intense M-70 fragment[12]. The carbonyl group in the three position has little effect on the fragmentation patterns of these compounds due to the influence of the C-17 side chain[17]. The stereochemistry of the methyl groups at C-4 and C-24 is unknown, although the 4α steroids are the dominant form found in nature[18]. On the other hand, 4β steranes have been found in oil shales[19]. No stanones unsaturated in the ring system were detected, nor were any 4,4-dimethyl stanones identified, although it seems from mass spectral evidence that some of these gem dimethyl compounds may be present at very low concentrations. The mono- and di-unsaturated 4-desmethyl sterenes[1] of both the saturated and unsaturated Δ22 series were detected and correspond to their side chain alkyl-substituted 4-desmethyl stanone counterparts.

The occurrence of 3-ketosteroids in biological systems is limited. 4α-Methyl-3-ketosteroids are known to be intermediates in sterol biosynthesis, but are only detectable in trace concentrations[20,21]. Hence, a planktonic or benthic faunal or floral source for sedimentary stanones is unlikely, although few studies have been undertaken in the marine environment[21]. It has been proposed that stanones are possible intermediates in one transformation pathway for the reduction of stenols to stanols in sediments[1,22]. The 4-desmethyl stanones isolated from surface sediments in Walvis Bay structurally correspond to their stenol precursors detected in these sediments (C. Lee, unpublished results) and in the overlying water column (R.B.G., unpublished results). Thus these stanones may represent a series of stenol oxidation products.

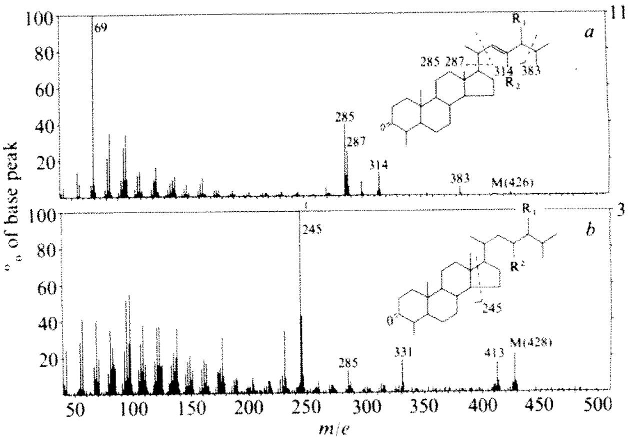

Fig. 3 Mass spectral line diagrams as obtained by glass capillary GC–MS of stanone fraction of core 16: *a*, 4,23,24-trimethylcholest-22-en-3-one (XII), $R_1{=}R_2{=}CH_3$ or 4-methyl, 24-ethylcholest-22-en-3-one, $R_1{=}H$, $R_2{=}CH_2CH_3$; and *b*, 4,23,24-trimethylcholestan-3-one (VI), $R_1{=}R_2{=}CH_3$ or 4-methyl, 24-ethylcholestan-3-one, $R_1{=}H$, $R_2{=}CH_2CH_3$. The mass spectra of these pairs of compounds are identical. Hence, a definitive structure proof cannot be made at present.

It is well known that 4-methyl steroids are important biosynthetic intermediates in the formation of 4-desmethyl steroids[20], although they are present in only small amounts in plants. Hence, another possible source for sedimentary 4-desmethyl stanones is from oxidation of the 4-methyl stanones followed by decarboxylation (Fig. 1)[20].

The 4-methyl stanones could arise from oxidation of 4-methyl stenols to 4-methyl stenones followed by hydrogenation to the corresponding stanone (Fig. 1). In this regard relatively large amounts of 4-methyl and 4,4-dimethyl sterols are known to accumulate in the bacterium *Methylococcus capsulatus*[23]. An array of 4-methyl sterols with varying degrees of alkylation and unsaturation in the side chain have been found in several plants[18]. It is also important that 4α,23,24-trimethyl-5α-cholest-22-en-3β-ol (dinosterol) a stenol precursor to one of the structures of (XII), was found to be the major sterol in the toxic dinoflagellate, *Gonyaulax tamarensis*[9].

As mentioned earlier, no 4-methyl or 4-desmethyl stenones were detected. These compounds are proposed intermediates in our hypothesised transformation of stenols to stanols (Fig. 1).

Their absence may be due to a more rapid rate of hydrogenation relative to their rate of formation from stenol oxidation. Hence their steady-state concentration would be quite low. Work is now being carried out on other sediment samples from both oxic and anoxic environments to elucidate further the proposed transformations outlined above. We emphasise, however, that the proposed mechanism in Fig. 1 is probably only one of several degradation pathways for sterols in sediments.

Pathways leading to the formation of sterenes from stenols have been proposed by several authors[1,22,24–28]. These transformations in Recent marine surface sediments can be envisaged as a series of oxidation, reduction, and dehydration reactions. The sampling site off the south-west coast of Africa is an area of intense upwelling and high productivity. The presence of known microbiological products[20,29] such as stanols[30] (C. Lee, unpublished results), stanones and sterenes[1] in the sediments and the rapid rate of sediment deposition in this sampling area suggests microbially mediated transformations. For example, cholest-4-en-3-one and 5α-cholestan-3-one have been proposed as intermediates in the microbial reduction of cholesterol to cholestanol[29]. However, little is known about the metabolism of marine bacteria, and much more information is required to understand more fully the processes influencing the transformation reactions of steroids in marine sediments.

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Geological evidence in favour of a Jotunheimen Caledonian suture

EARLY plate-tectonic reconstructions of the proto-Atlantic by Wilson[1] and Dewey[2] placed a Caledonian suture through central, southern Norway. Later, more detailed interpretations generally favoured a single (proto-Atlantic, Iapetus) suture off the west coast[3–14], in agreement with the pre-plate-tectonic orthodoxy[15–18] of a western, eugeosynclinal source for the far-travelled (up to 1,000 km) (ref. 13), crystalline and supracrustal nappes of Norway and Sweden. In South Norway, however, not all interpretations fitted this pattern. Goldschmidt[21] and Oftedahl[22] proposed a local root zone in the Jotunheim for the crystalline Jotun Nappe south-east of its present northwesterly margin. More recently Battey and McRitchie[23,24] demonstrated a close fit between surface geology and the interpretations of gravity data by Smithson *et al.*[25] of the Jotun Nappe as an 'Ivrea type' flake with a local root possibly as deep as 16 km. Note that recent[43] aeromagnetic results suggest a maximum thickness of only 5 km for the Jotun Nappe). Evidence from the northwestern margin of the Jotunheim is clearly critical, for the south-eastward transport of the Jotun Nappe deduced from areas to the south-east[14,18] is consistent with derivation from both the Iapetus suture and a possible Jotunheim suture. Some reports, however, apparently support the Jotunheim suture hypothesis. Between Fjaerlandsfjorden and Sogndalsdalen, Skjerli[26] found imbricated thrust slices of Basal gneisses and supracrustal rocks indicating transport to the north-west. A similar conclusion was reached by Battey and McRitchie[23] for Bøverdalen. Also, Elliott and Cowan[27] describe a sequence of tholeiitic greenstones with pillows and serpentinites from Holleindalen in Bøverdalen. Most recently, however, Roberts[42] has concluded, using structural evidence from seven traverses across the northwestern margin, that the Jotun Nappe was transported to the south-east. The work described here does not support this conclusion.

Within the Sygnefjell area (Fig. 1) an Assynt-type nappe culmination permits the examination of an extensive sequence of supracrustal rocks between the Basal gneisses and the overlying Jotun Nappe. Six main lithostructural units have been identified:

6	Jotun mangerites and anorthosites	Jotun Nappe; allochthon
5	Galdeberghytta gneisses	
4	Helgedalen metagreywackes	Parautochthon?
3	Turtagrø metavolcanics	
2	Sygnefjell sparagmites	
1	Basal gneisses	Autochthon

The Basal gneisses form part of the extensive northwestern Basal gneiss complex of South Norway. An early suite of

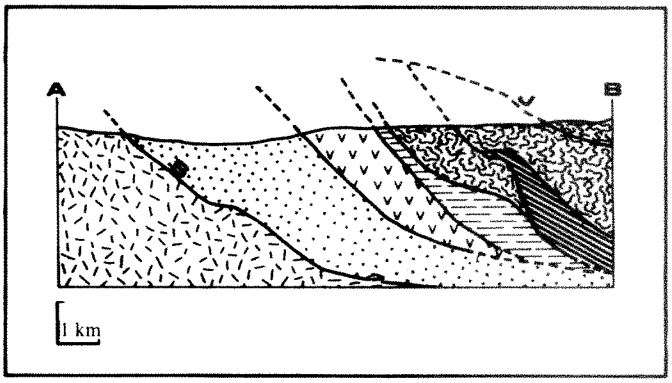

Fig. 1 A simplified lithostructural map of the Sygnefjell area: 1, Basal gneisses; 2, Sygnefjell sparagmites; 3, Turtagrø metavolcanics; 4, Helgedalen metagreywackes; 5, Galdeberghytta gneisses; 6, Jotun mangerites and anorthosites. St, Storevatnet; Sk, Skaalavatnet; Pr, Prestesteinsvatnet; H, Helgedalen; G, Galdeberghytta; K, Krossbu.

basic–intermediate rocks has been migmatised and metamorphosed in the almandine–amphibolite facies and intruded by foliated granites dated at 1,033 Myr (refs 28, 29).

Micaceous meta-arkoses (sparagmites) have, in places, a basal conglomerate resting unconformably on the Basal gneisses. They are intercalated with metalimestones, graphitic micaschists and hornblende schists higher in the sequence.

The Turtagrø metavolcanics comprise some 600 m of andesitic and basic lavas, some with pillows, and tuffs within a thrust bounded slice. This is overlain by up to 1 km of Helgedalen metagreywackes. The greywackes grade from a proximal, quartz-rich, conglomeratic facies at the base, to a distal, finegrained, apparently volcaniclastic facies towards the top. Graded bedding and various other sedimentary structures indicate deposition by turbidity currents, and provide evidence that the unit as a whole is the right way up.

All supracrustal units have been recrystallised in the greenschist facies. The absence of fossils or radiometric dates precludes a reliable assessment of their ages, although regional considerations[18,42] suggest an Eocambrian to Lower Ordovician age. Despite the thrusts within and between these supracrustal units, all are at present regarded as parautochthonous in view of the apparent lithological gradations between them. Moreover, all these units are intruded by pre-kinematic, minor gabbros, dolerites and diorites.

The Galdeberghytta gneisses at the base of the Jotun Nappe are partially mylonitised, amphibolitic and augen gneisses with K-feldspar-rich, late pegmatites and minor intrusives. The upper part of the Jotun Nappe consists of layered gabbros, leucogabbro, anorthosite and mangerites in various stages of retrogression from granulite facies, pyroxene-bearing assemblages. Battey and McRitchie[23] cite K–Ar age determinations of up to 1,280 Myr for these rocks to the south-east of the Syngefjell area.

Structural work has shown that both the Basal gneisses and the Jotun Nappe rocks were deformed and metamorphosed before the events that affected the supracrustal sequence. The supracrustal rocks share a common polyphase structural sequence. A transposed tectono-metamorphic banding was developed to varying degrees in all units during early deformations (D_1 and D_2). Where younging criteria can be identified, the D_1 folds face the north-west, suggesting relative transport in that direction. Intense flattening in the limbs of the D_2 isoclinal folds has resulted in discrete zones of mylonitic rocks. Following D_2, a phase of upright asymmetric folding (D_3) with the steep limbs overturned to the north-west took place syntectonically with major brittle thrusting (D_4) of all units (Fig. 2). The thrusting shows classical imbrication patterns, with the thrusts climbing section towards the north-west, indicating transport in that direction.

A similar structural geometry and history of folding has been described by Hopper (in preparation) from Østerøy near the northeastern margin of the Bergen Arcs, and by Cowan[31] from Bøverdalen. The Vassenden area, Bøverdalen, is one of those recently described by Roberts[42]. Both Cowan's (ref. 31 and personal communication) earlier work at Vassenden, and evidence from the nearby Sygnefjell area, indicate that a major phase of structures, including north-west facing folds (our D_1), pre-dated the D_1 of Roberts. Thus, in our view, the Vassenden area does not provide good evidence for nappe transport from the north-west.

An outline of the sequence of events in the Sygnefjell and nearby areas together with suggested broader interpretations can now be given.

First, pre-Sveconorwegian[23,29,32] high grade metamorphism and deformation of Jotun Nappe rocks (and of the north-west Basal gneisses and southern gneisses of other areas[29,34,38]).

Second, Sveconorwegian (1,033 Myr) intrusion of Hestbrepiggan granite into Basal gneisses.

Third, post-1,033 Myr (Eocambrian to Lower Palaeozoic?): (1) deposition of a fining-upwards sequence of conglomerates, limestone and shale; (2) sub-aqueous basaltic volcanism (ocean spreading); (3) andesites and turbiditic, volcaniclastic greywackes (island arc–trench system); (4) greenschist facies metamorphism and deformation of supracrustal wedge (ocean closure); (5) thrusting of the Jotun Nappe to the north-west (after flaking from lower crust during final stages of ocean closure); (6) broad warping and sinistral wrench faulting (after ocean closure).

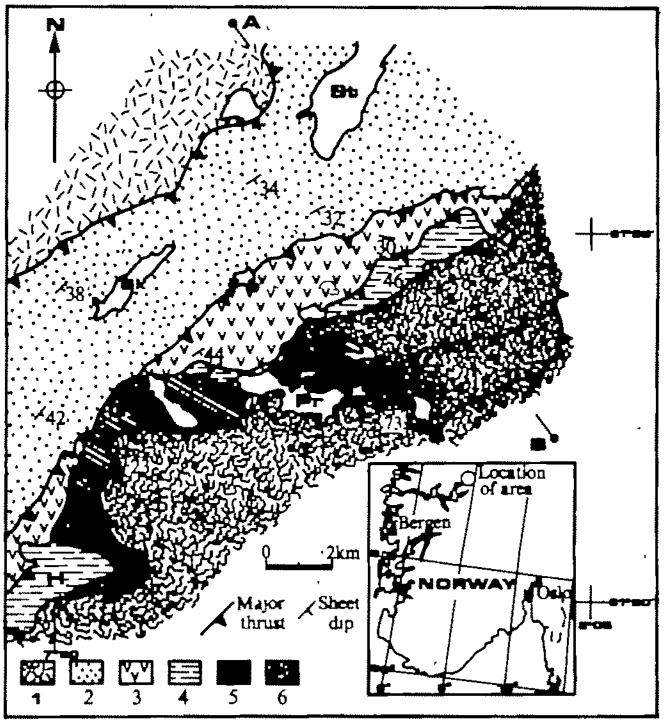

Fig. 2 A simplified dip section of the Sygnefjell area; the area has an along-strike relief of 1 3 km. J, Jotun Thrust; S, Sole Thrust; A–B, ornament as on Fig. 1.

Long distance southeastward transport of the Jotun Nappe has been suggested in the past for two main reasons. First, on the similarity of the Jotunheim and Bergen Arc high-grade metaplutonic rocks. Deep crustal granulite facies rocks are not uncommon in old basements, however, and many Jotun Nappe rocks can be matched in the basements both to the north-west and to the south-east of Jotunheimen (see refs 33, 39, 40). In any case, lithological similarity alone cannot indicate the direction of nappe transport.

Second, the occurrence in the nappe region of east to southeast trending penetrative lineations has been taken (see ref. 16) to indicate a southeastward direction of nappe transport. In the Sygnefjell area the early, penetrative lineation is due to the superimposition of at least two strains (D_1 and D_2) and a transport direction cannot be deduced from this evidence alone. Similar conclusions have recently been reached in the Bergen area by Faerseth *et al.*[41] and one of us (F.W.M.H., in preparation).

Within the Sygnefjell area, two groups of observations are consistent with the hypothesis of a Jotunheim suture:

First, the facing directions of the early folds (D_1), and, especially, the thrust geometries produced during the main movement phases (D_4), indicate tectonic transport to the north-west.

Second, the occurrence of a probably parautochthonous, oceanic, supracrustal sequence of arkoses, turbiditic greywackes, serpentinites and basaltic and andesitic volcanics.

On the other, southeastern margin of Jotunheim, it is well-established that both the supracrustal and the crystalline nappes were transported towards the south-east. That the supracrustal nappes moved considerable distances (100–270 km) is shown by Hossack's[14] recent unravelling of the thrust stack. The even greater distance of transport (290+km) 'assumed' (ref. 14 p 239) for the crystalline, Jotun Nappe above, although consistent with evidence from the supracrustal nappes, is not required by it, as Hossack clearly recognises. When the Sygnefjell evidence is also considered, a possible interpretation emerges of the Jotun Nappe as a flake detached from local, lower crust and thrust over (underthrust by) gneissose basements and marginal sedimentary basins both to the north-west (such as Sygnefjell) and to the south-east (such as Bygdin). The presence of igneous rocks within the northwestern supracrustals may indicate their proximity to the site of an active trench.

Further work will test the hypothesis of a Jotunheim Caledonian Suture. The structural evidence reported here will be published in full elsewhere.

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Mechanism of reaction in metal + metal oxide systems

PYROTECHNICS frequently consist of intimate mixtures of powdered metals and metal oxides. The exothermicity of such reactions is often very high, as, for example, in the thermite process. Considerable self-heating occurs and the reaction often becomes explosive. Little experimental work has been carried out on the mechanism of these reactions, largely because the participation of solid phases and the high evolution of heat make them difficult to study. It is to be expected, however, that such mechanisms would normally involve solid phases, with the possible addition of a liquid phase when a low melting point reactant or intermediate is present. We report here that such reactions often occur through a gas phase process. Furthermore, when one of the constituents is magnesium, which is frequently the case in pyrotechnics, the reaction rate is controlled almost solely by the evolution of magnesium vapour.

The classical method, developed by Wagner[1] and by Tubandt[2–4], for elucidating the identity of the mobile species and of any intermediate compounds formed, is to allow pressed pellets of the two separate solids to react together. The movement of the phase boundaries and the growth of intermediate zones of different composition then provide the required information. During preliminary studies on metal + metal oxide systems, using this technique, it was discovered that reaction proceeds normally even when there is a substantial gap between the two solids, and the system is well below the melting point of the materials involved. This demonstrated that the mobile species was present in the gas phase and the chemical reaction involved both gaseous and solid components. A more comprehensive series of experiments was then carried out.

A typical reaction chamber consisted of a pair of mild steel 'crucibles', each of 8 mm internal diameter and 8 mm internal depth. The reactant samples were ground and sieved to the required particle size and quantities of about 0.2 g were packed into each crucible to the required pressure. The crucibles were heated separately *in vacuo* to remove occluded gases and were then handled only in dry, O_2-free conditions. After weighing, the crucibles were clamped together so that the reactant surfaces were about 2 mm apart and the complete reaction chamber was heated in a thermostatted oven, either *in vacuo* or in a low pressure of argon, for the required period (typically 1 h). At the end of the experiment, the two crucibles were separated and re-weighed to determine the mass of material transported (typically ~40 mg, corresponding to 2 mmol of reaction or 20% conversion). The product layers were then separated manually for analysis. Blank experiments were also carried out on the individual components.

The main results are shown in Table 1, from which the most

Table 1 Summary of metal + metal oxide reactions

System	$\Delta H^0_{f,300}$ of oxide (kcal mol^{-1})	$\Delta H^0_{f,300}$ per atom of oxygen	Temperature of reaction (°C)	Mobile species	Products identified
$Mg+SiO_2$	210	105	625	Mg	MgO, Si, Mg_2Si
$Mg+Cr_2O_3$	275	91	625	Mg	MgO, Cr, $MgCr_2O_4$
$Mg+MoO_2$	133	66	625	Mg	MgO
$Mg+WO_2$	137	69	625	Mg	
$Mg+B_2O_3$	302	101	1000*	Mg	MgO, MgB_2
$Mg+Al_2O_3$	404	135	625	Mg	MgO, Al
$Mg+TiO_2$	228	114	625	Mg	MgO, Ti
$Mg+CuO$	37.7	37.7	625	O	MgO, Cu
$Mg+NiO$	57.5	57.5	625	O	MgO, Ni
$Ti+Cr_2O_3$	275	91	1300		no reaction
$Ti+NiO$	57.5	57.5	900	O	Ni, TiO_2

The heat of formation, $\Delta H^0_{f,300}$, for MgO = 144 kcal mol^{-1}.
* The amount of reaction at lower temperatures was negligible.

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important conclusion is that, in the majority of cases involving magnesium, which has a vapour pressure of ~2 torr at these temperatures, the mechanism simply involves evaporation of magnesium from the solid followed by reaction at the oxide surface, in one or more stages. In such cases, the rate is determined primarily by the rate of evaporation of magnesium, an exception being the reaction with boric oxide.

When the oxide is less stable, as indicated by a lower heat of formation, the mobile species becomes oxygen. However, the mechanism involved is not simple dissociation of the oxide, for the following reasons. (1) In the absence of the metal reactant, the weight loss from the oxide was negligible (as would be predicted from the dissociation pressures). (2) In the presence of a non-volatile metal, the reaction was much slower and required a substantially higher temperature. (3) The extent of reaction was comparable with that of systems in which magnesium was transported. (4) Some of the magnesium oxide (~5%) was detected on the Ni/NiO surface.

It seems, therefore, that the dissociation of the oxide to give mobile oxygen is 'catalysed' by the presence of metal, presumably due either to the considerable heat evolved on reaction, thus raising the temperature of the oxide surface, or to the formation of intermediates of different composition.

Attempts to compare the kinetics of reaction using this technique were of only limited value for two reasons: first, the initial rate of evaporation of magnesium depended very much on its pretreatment and was thus rather irreproducible, and second, because the rate decreased markedly with time, due presumably to inhibition by solid product, the extent of reaction tended to be similar even when the initial rates differed. Quantitative kinetic studies will require a controlled and measurable pressure of magnesium vapour.

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Photosynthesis in some British coastal waters may be inhibited by zinc pollution

RIVER drainage from mineral-bearing and industrialised regions has produced elevated concentrations of heavy metals in several parts of the coastal waters around the UK, including Liverpool Bay[1,2], Cardigan Bay[1], the Bristol Channel[1-3], off the mouth of the River Tees[4] and in the Firth of Clyde[5]. Little attention has been paid, however, to the possibility that the presence of the metals might be affecting the planktonic marine life of these sea areas. Phytoplankton represent the basic food resource on which the majority of marine animals and fish directly or indirectly depend, and it is thus conceivable that inhibition of their growth by toxic metals could have repercussions throughout the local marine food webs. We are investigating the role of trace quantities of both biologically beneficial and toxic substances in regulating the 'quality' of seawater, that is, its capacity to support and maintain, through photosynthesis, the production of living matter from the inorganic nutrients present in the water. As part of this research programme, we have examined how carbon fixation rates in natural populations of phytoplankton are affected by zinc concentrations encompassing those observed in the areas mentioned. Although zinc is a component of many enzyme systems[6] and therefore essential for the growth of phytoplankton[7], it becomes toxic if present greatly in excess of their basic requirements. We report here data which suggest that phytoplankton growth could be depressed by the zinc concentrations present in some British coastal waters.

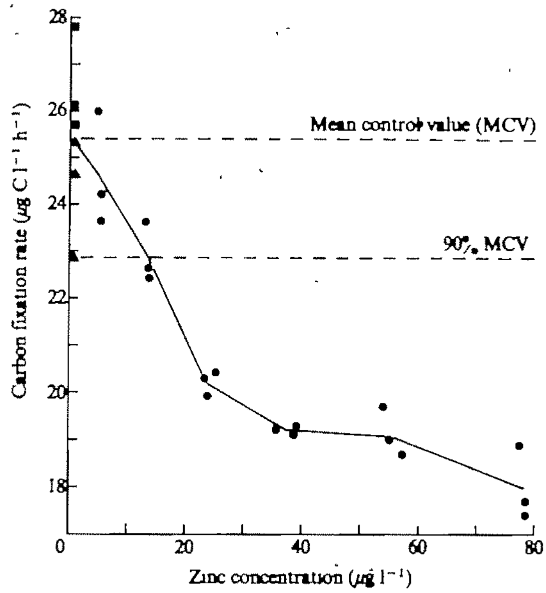

Fig. 1 The effect of increasing concentrations of zinc on the rates of carbon fixation in the phytoplankton present at Station L4 (50°15′ N, 4°13′ W) in the English Channel on 10 July 1978. The lines connect the mean values of each group of results. The measurements were carried out at 14 °C and 10 klx of illumination by Northlight fluorescent lighting. Sample 1 contained 2 1 µg chlorophyll *a* per l, the phytoplankton being almost exclusively diatoms of the genus *Rhizosolenia*. The chlorophyll *a* concentration has been corrected for the presence of phaeo-pigments ▲, ■, Controls.

Full details of the techniques being used will be published elsewhere. Briefly, samples of surface seawater, strained through a 170-µm nylon mesh to remove the zooplankton, were rapidly transported from the point of collection—Station L4 about 10 km south west of Plymouth breakwater—to our laboratory. During the journey, the samples were kept at seawater temperature and exposed to natural lighting conditions. Immediately after their arrival in mid-afternoon, zinc labelled with ^{65}Zn was added to eight 1-l subsamples of the water to give initial concentrations ranging from those present naturally (in the two controls) up to about 90 µg l^{-1}. The subsamples were then placed in an illuminated incubator maintained at the temperature of the seawater at the time of collection and left there overnight to allow the added zinc to equilibrate with the plant cells. The water was aerated throughout the whole of this period in the laboratory to maintain a constant pH and keep the phytoplankton in suspension. Between 20.00 and 06.00 h, the lights were extinguished to simulate overnight darkness. The next day, the carbon fixation rates of the phytoplankton in each of the subsamples were determined in triplicate in the illuminated incubator by measuring the rate of incorporation of $^{14}CO_2$ (ref. 8). Discrimination between the β and γ radioactivities due to the ^{14}C and ^{65}Zn, both in the water and in the phytoplankton (after their removal from the water by membrane filtration), allowed the carbon fixation rates and the amounts of zinc taken up by the plant cells (expressed as µg Zn

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per µg chlorophyll *a*) to be determined as functions of the concentration of zinc present in the water during the carbon fixation measurement. The zinc concentration at this stage of the experiment was always lower than that initially, due to adsorption on to the glassware used to contain the water samples.

Figures 1 and 2 show the results of the measurements on one of the samples. Two further samples collected on successive weeks contained phytoplankton populations consisting of approximately equal numbers of dinoflagellates (mainly *Scrippsiella* aff. *trochoidea*) and diatoms (mainly *Rhizosolenia* spp.); carbon fixation rates in these were similarly depressed by the presence of zinc. In a control experiment, the normal routine was carried out with the exception that zinc was not added to any of the eight subsamples. In this case, the carbon fixation rates in each of the 24 final determinations agreed to within ± 10% of the mean value. In Fig. 1, fixation rates less than 90% of the mean control value can thus be regarded as due to perturbations caused by the zinc.

The results show that zinc concentrations down to 15 $\mu g\, l^{-1}$, and possibly lower, inhibited photosynthesis in the phytoplankton populations present in the English Channel in July 1978. The degree of inhibition was, however, a nonlinear function of the zinc concentration and above about 30 $\mu g\, l^{-1}$, there was very little further decrease in the carbon fixation rates. This was apparently due to the near saturation of the plant cells with zinc at the higher concentrations, as the zinc/chlorophyll *a* ratios in the phytoplankton were then approaching their maximal values (Fig. 2); the forms of these zinc sorption isotherms were found to differ from one sample to the next, probably due not only to the change in the species composition of the phytoplankton present in the water but also, to some extent, to uptake on to detrital matter which it was not possible to separate from the plant cells. These observations support the recently expressed view[9] that the effect of metals on the growth of phytoplankton is more closely related to the amounts accumulated by the plant cells than to the concentrations in the water surrounding them.

In the contaminated sea areas, dissolved zinc concentrations between 5 and 10 $\mu g\, l^{-1}$ were found to be widespread and at many locations, sometimes greatly, exceeded 10 $\mu g\, l^{-1}$ (refs 1–3, 5). Thus, it seems possible that primary production in these regions may already have been depressed as the result of metal pollution, particularly when it is remembered that zinc was just one of a range of metals usually present in the water[1–5], and synergistic effects as observed with zinc and copper mixtures in laboratory cultures[10] could amplify their individual toxicities towards the phytoplankton.

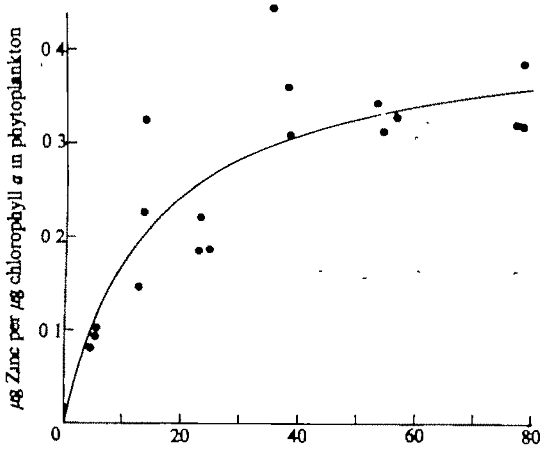

Fig. 2 Zinc/chlorophyll *a* ratios in the phytoplankton during measurement of the carbon fixation rates in relation to the zinc concentration in the seawater. The curve represents the best fit of the Langmuir adsorption isotherm $y = Ax/(B+x)$ to the data, the values of A (µg Zn per µg chlorophyll *a*) and B (μg Zn l^{-1}) used to compute it being 0.42 and 14.7

However, because our measurements were short term and relate to phytoplankton growing in the relatively unpolluted waters of the English Channel, extrapolation of the present data to other sea areas can only be made with some reservations. First, the metal contamination is generally associated with river drainage so that the polluted seawater is also likely to contain higher than usual levels of dissolved organic matter[11]. The formation of metal–organic complexes would decrease the biological availability of the metals and hence the amounts taken up by the phytoplankton, thereby moderating their effects in the way that chelating agents reduce the toxicity of copper in phytoplankton cultures[12]. Second, chronic exposure of metal-sensitive phytoplankton populations to sublethal metal concentrations might eventually lead to their replacement by tolerant species or the development of resistant strains[13] which would be unaffected by the presence of toxic metals. Nevertheless, the existence of potentially harmful zinc concentrations in our coastal waters clearly requires further investigations.

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Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium

IN marked contrast to their freshwater counterparts, marine planktonic cyanobacteria are restricted to a few nostocalean genera, of which only *Trichodesmium* is capable of forming extensive water blooms[1–3]. We report here the widespread occurrence of a small, marine, chroococcalean cyanobacterium belonging to the genus *Synechococcus*.

Natural water samples were filtered through 0.2 µm Nuclepore filters, counterstained with Irgalan black[4]. The filters were examined with a Zeiss Standard microscope equipped with Neofluar objectives and an epifluorescent illumination system containing a 100-W halogen lamp, a BP 450–500 excitation filter, a LP 528 barrier filter and a FT 510 chromatic beam splitter. Using this system, phycoerythrin-containing cyanobacteria fluoresce orange and can be distinguished from phytoplankters that fluoresce red.

Phycoerythrin-rich unicellular cyanobacteria were observed at seven stations in the Arabian Sea in January 1977, at three stations off the coast of Peru in March 1978, in Slope Water north of the Gulf Stream in April 1978, and periodically in Woods Hole Harbor. In the relatively rich waters of the Arabian Sea and off the coast of Peru, the population varied from 10^4 to 10^5 cells ml^{-1} within the euphotic zone (Table 1). The greatest number of cells was found within the top 20 m of the water column, with occasional cells being observed as deep as 400 m. In contrast, the surface sample collected from Slope Water north

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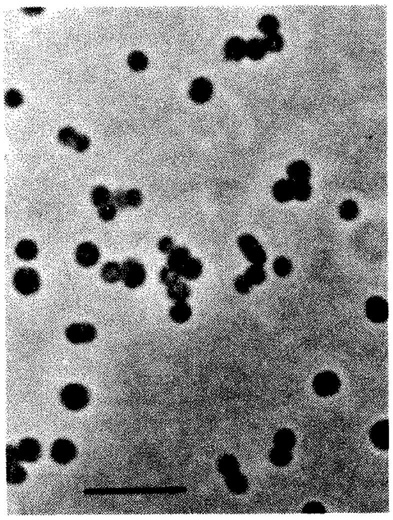

Fig. 1 Phase contrast photomicrograph of *Synechococcus* sp. (strain Syn-48) illustrating general cell morphology (scale bar, 5.0 μm).

of the Gulf Stream contained 1.3×10^3 cells ml^{-1}. In Woods Hole Harbor, counts have ranged from 2×10^3 cells ml^{-1} on 1 May 1978 to 3.6×10^5 cells ml^{-1} on 1 June 1978, at which time 15–20% were in a late stage of binary fission.

No attempt was made to culture the cyanobacteria from the above locations. However, in 1965, one of us (R.R.L.G.) isolated a small, red, coccoid cyanobacterium (strain Syn-48) off the north-east coast of South America (08°44′ N, 50°50′ W) with morphological and fluorescent properties similar to the cyanobacteria observed in open ocean waters by epifluorescence microscopy. Its size is $0.9–1.3 \times 1.8–2.2$ μm and it divides in one plane (Fig 1). Examination of thin sections by transmission electron microscopy reveals that it has a typical synechococcoid ultrastructure, with the thylakoids arranged peripherally. H. W. Siegelman (personal communication) reports that its pigment system is typical of other cyanobacteria, containing chlorophyll *a* as its major photosynthetic pigment and phycobiliproteins as accessory pigments. It always appears red in culture, due to both a predominance of phycoerythrin, one of the accessory photosynthetic pigments, and its inability to adapt chromatically[5,6]. The strain was isolated from a depth of 75 m where the salinity was 35.87‰. Preliminary growth experiments, carried out as described by Hargraves and Guillard[7], reveal that it will not grow at salinities below 14‰, indicating that it is intrinsically marine[8,9].

Recently, several additional strains of small, red, coccoid cyanobacteria have been cultured from Georges Bank and the Sargasso Sea. In general, they resemble both strain Syn-48 and the cells observed by epifluorescence microscopy. Individual isolates vary from 0.8 to 2.0 μm in diameter following binary fission.

Table 1 Representative vertical distributions of *Synechococcus* sp.*

Depth (m)	Station 28 Peru 13°52′ S, 76°34′ W	Station 30 Peru 13°57′ S, 77°10′ W	Station 2354 Arabian Sea 21°16′ N, 68°05′ E	Station 2356 Arabian Sea 24°12′ N, 64°44′ E
0	1.90	8.80	12.00	5.30
10	2.60	1.08	12.50	
20	0.48	1.04	4.28	
25				5.28
40	0.024	0.076		
50			6.72	3.95
60				
75			6.08	
100		0.16	3.00	0.016
150			0.016	
200		0.026	0.013	0.013
300				0.011
400			0.010	<0.001

* 10^4 Cells per ml.

Cell size and shape accompanied by division in one plane indicate that these cyanobacteria are assignable to the genus *Synechococcus*[10], an assignment which is further substantiated by the typical synechococcoid ultrastructure of strain Syn-48. The range in cell size among the isolates now in culture suggests that they may represent more than one species.

The use of epifluorescence microscopy to visualise the natural orange fluorescence of phycoerythrin seems a useful technique to enumerate small, phycoerythrin-containing unicellular cyanobacteria. However, not all cyanobacteria contain phycoerythrin, so that the specific set of filters used in this study cannot be used to count unicellular cyanobacteria in general. Furthermore, phycoerythrin is not unique to cyanobacteria but is present in almost all red algae (Rhodophyta) and in many cryptomonads (Cryptophyta). In principle, members of the latter two groups could interfere when attempting to count unicellular cyanobacteria, but in practice differences in size and chloroplast morphology should permit the unambiguous distinction between these eukaryotic algae and unicellular cyanobacteria.

Cyanobacteria have an ancient marine history[11] and show a considerable diversity and abundance in other marine habitats (for example, the intertidal zone)[1,2]. The existence of small unicellular cyanobacteria with a widespread geographical distribution and the ability to achieve considerable cell densities represents the second example of a planktonic cyanobacterium that has adapted to growth in the open ocean and the first well documented member of the Chroococcales to do so. The ecological importance of these cyanobacteria as major primary producers in the world's oceans is still to be assessed.

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Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*

NATURAL SELECTION, in sexually reproducing plants, should often favour matings between individuals of intermediate genetic similarity. Matings between very similar individuals may lead to inbreeding depression because segregational load is revealed[1,2], while matings between very dissimilar individuals may disrupt favourable gene combinations and lead to outbreeding depression[3-5]. Outbreeding depression in plants has

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been documented in crosses between species, varieties and isolated populations[6–9], and reports of inbreeding depression date back at least a century[10]. We suggest that outbreeding depression will often occur on a much finer scale than previously recognised, especially in plants subject to restricted pollen and seed dispersal. Such plants are likely to show pronounced microgeographic genetic differentiation resulting from drift in subpopulations isolated by distance or from adaptation to local edaphic and biotic conditions[11,12]. Under these circumstances, a short outcrossing distance may be optimal not only because of intragenotypic effects, but also because it produces offspring sufficiently similar to the female parent to grow successfully near her, yet sufficiently genotypically diverse to maximise success of the total progeny in the face of coarse-grained temporal environmental variation[13–15], frequency-dependent sibling competition[16–18] or predation[19–20]. Here we present evidence that a short outcrossing distance is optimal for *Delphinium nelsoni* Greene and discuss the relationship between the optimal outcrossing distance for *D. nelsoni* and actual pollen dispersal by its main pollinators.

D. nelsoni is an herbaceous perennial which flowers in early summer around the Rocky Mountain Biological Laboratory (RMBL) in western Colorado[21]. It lacks clonal spread, the flowers are usually borne 10–20 cm from the ground and seeds drop an average of 11.2 cm from the central stalk. To investigate the effects of outcrossing distance on fitness, we hand-pollinated 1,016 flowers on 317 representative *D. nelsoni* plants near RMBL in 1976, 1977 and 1978. In the 1976 Meadow 1 and 1977 Meadow 2 experimental replicates, we bagged recipient plants to exclude natural pollinators and chose pollen donor flowers either from the same plant (self pollination) or from plants 1, 10, 100 or 1,000 m distant. In the 1977 Meadow 1 replicate we omitted 1 and 100 m treatments, while in the 1978 Meadow 1 replicate we substituted 3 and 30 m for selfed and 1,000 m treatments. Different pollen donor populations were used for Meadows 1 and 2, which are separated by 300 m of aspen forest and differ by 60 m elevation. We assigned each plant at random to a single distance treatment to avoid systematic bias in plant size or ovule number per flower, and used individual pollen loads from that distance to pollinate several of its flowers as they became receptive. Pollen loads were collected on circular toothpicks, usually from one donor flower but where dehiscing anthers were scarce from as many as three flowers on two plants. This collection technique was consistent between treatments within a replicate. In the 1977 Meadow 2 and 1978 Meadow 1 replicates, we equalised the amount of mechanical handling of pollen during transport from the donor population and the total elapsed time between its collection and deposition across treatments. We harvested fruits on recipient plants just before dehiscence and counted all fully developed seeds. Our hand pollination procedure consistently resulted in seed sets of approximately 15–35% of open-pollinated averages[21]. In all replicates, we left several flowers per plant unpollinated to serve as controls for failure of bags to exclude pollinators. We discarded the few plants in which these control flowers set copious seed, as *D. nelsoni* is protandrous and rarely sets seed when pollinators are effectively excluded[21]. In the 1976 Meadow 1 replicate, we lumped all mature seeds for each treatment and planted them in a 10 m^2 plot near Meadow 2. In the 1977 Meadow 1 replicate we lumped mature seeds from each plant and planted each progeny separately within a 2 m^2 plot in the center of Meadow 1. These plots were censused in early June during subsequent summers to determine seedling survivorship.

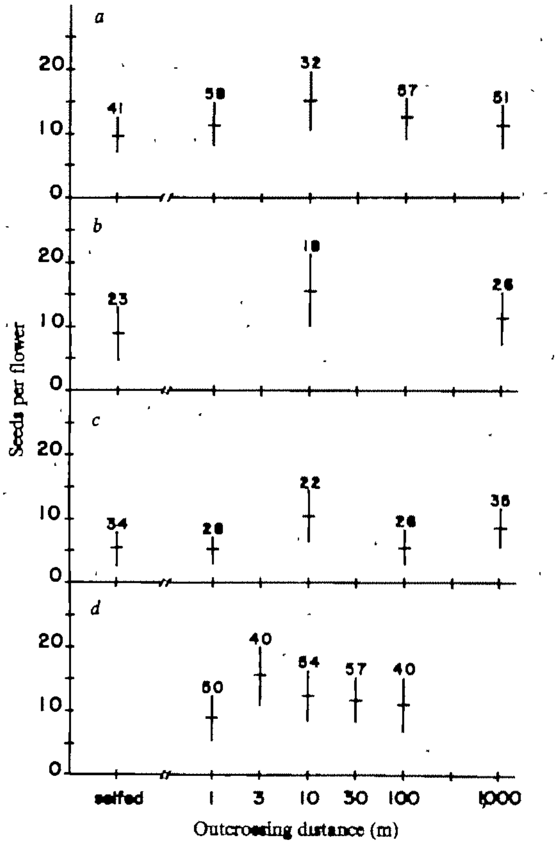

Fig. 1 Effects of outcrossing distance on seed set in hand-pollinated *D. nelsoni*. *a*, Meadow 1, 1976; *b*, Meadow 1, 1977; *c*, meadow 2, 1977; *d*, meadow 1, 1978. Values are means ±95% confidence units, with sample sizes shown. Two statistical tests establish significance of the intermediate outcrossing optimum. First, for the three 1976–77 experimental replicates, 10 m seed sets were significantly higher overall than either 1,000 m (d.f. = 6, $\chi^2 = 12.59$, $P < 0.05$) or selfed (d.f. = 6, $\chi^2 = 24.20$, $P < 0.005$) seed sets, using combined probabilities from separate one-tailed *t* tests with each replicate[22]. Second, the probability of observing by chance alone an intermediate optimal outcrossing distance in the 1976 replicate, followed by peaks at the same distance interval for the remaining three replicates is $3/5 \times 1/3 \times 1/5 \times 3/5 = 0.024$

Table 1 Effects of outcrossing distance on seedling survivorship in *D. nelsoni*

	Treatment				
	Selfed	1 m	10 m	100 m	1,000 m
1977 Meadow 1 seeds, planted in Meadow 1, censused after 1 yr					
No. planted	58	—	145	—	116
No. surviving	3	—	23	—	13
Proportion surviving	0.052	—	0 159	—	0.112
1976 meadow 1 seeds, planted in Meadow 2, censused after 2 yr					
No. planted	98	98	98	98	98
No surviving	0	3	7	1	1
Proportion surviving	0	0.031	0.071	0 010	0 010

A significantly higher proportion of 10 m seeds survived overall than either 1,000 m (d f. = 4, $\chi^2 = 13.47$, $P < 0.01$) or selfed (d.f = 4, $\chi^2 = 28\ 30$, $P < 0\ 005$) seeds, using combined probabilities from separate one-tailed *t* tests of differences of proportions with each replicate[22].

For all hand pollination experiments mean seed sets per flower were highest with an intermediate outcrossing distance and declined significantly at longer and shorter distances (Fig. 1). The three 1976–77 replicates consistently indicate an optimal outcrossing distance of 1–100 m, whereas the 1978 replicate more precisely indicates an optimal distance between 1 and 10 m. Note that in Meadow 1 mean seed sets for each treatment were especially similar between years. An optimal outcrossing distance between 1 and 100 m again appeared in seedling survivorship in natural meadows, and inbreeding and outbreeding depressions in survivorship were also significant (Table 1).

The main pollinators of *D. nelsoni* in the RMBL area are broad-tailed hummingbirds (*Selasphorus platycercus*), occasional rufous hummingbirds (*S. rufus*) and bumblebees (primarily *Bombus appositus* and *B. flavifrons* queens)[21]. Pollen dispersal by these visitors was estimated in two ways in 1976: (1) Foraging pollinators were followed in Meadows 1 and 2, with observations of bees and birds matched by meadow, day, and

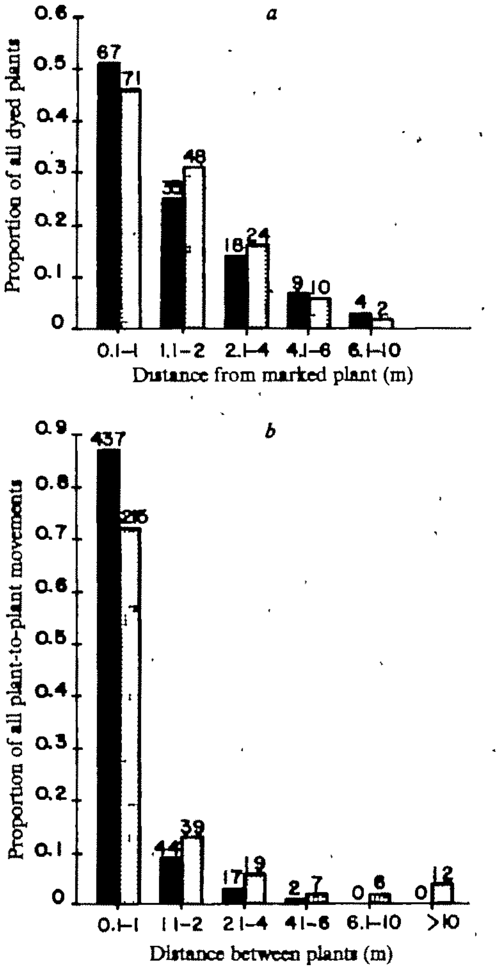

Fig. 2 Estimates of pollen dispersal by pollinators of *D. nelsoni*. *a*, Distributions of dye dispersal distances for caged (■)($\bar{x}$ = 1.64 m) and uncaged (□)($\bar{x}$ = 1.57 m) plants, with sample sizes (numbers of neighbouring plants receiving dye) shown *b*, Distributions of plant-to-plant movement distances for hummingbirds (□)(from 19 foraging bouts $\bar{x}$ = 2.1 m) and bumblebees (■)(from 12 foraging bouts $\bar{x}$ = 0.63 m), with sample sizes (number of movements) shown above each histogram.

time of day, and numbers of flowers visited per plant and distances flown between successive plants were noted. Densities in these meadows averaged 16 plants per m^2. (2) Dispersal of fluorescent pigments dusted on 18 plants in Meadow 2 was measured. Half of these plants were caged with chicken wire to exclude birds but not bees, and half were exposed to both pollinators. Caged and uncaged plants were dusted in pairs with distinct colours over a period of 2 weeks. Three to five days after dusting, neighbouring plants within a 10 m radius were sampled and scored for presence of pigment grains on floral parts contacted by pollinators.

Figure 2 compares distributions of plant-to-plant movements of hummingbirds and bumblebees and patterns of pigment dispersal from caged and uncaged plants. On average, birds moved 3.4 times as far as bees, but pigment moved no further from uncaged plants (exposed to birds as well as bees) than from caged plants. Movement distributions for bees and birds differ significantly overall ($\chi^2 = 21.0$, d.f. = 5, $P > 0.005$), while those for pigment from caged and uncaged plants do not ($\chi^2 = 2.5$, d.f. = 4, $P > 0.5$). Movement distributions for both pollinators are more highly leptokurtic than those for pigment, perhaps as a result of pigment carryover past the first recipient flower.

Expressing pollinator foraging only in terms of plant-to-plant movements conceals the fact that hummingbirds visit more flowers per plant than do bumblebees (birds, 2.5 ± 0.09 flowers per plant (296); bees, 1.4 ± 0.06 flowers per plant (502); values are means ± one s.e.m. followed by sample sizes). Counting within-plant movements as 0 m, mean flower-to-flower distances flown by the two pollinator types are more similar than mean plant-to-plant distances (birds, 0.8 ± 0.13 m between flowers (741); bees, 0.4 ± 0.03 m between flowers (731). Within-plant movements of hummingbirds are also especially likely to lead to selfing: birds appear to move unsystematically between flowers on an inflorescence, whereas our observations suggest that bees usually visit lower, pistillate flowers before moving up an inflorescence to staminate flowers[23,24].

The mean pollen dispersal distance estimated by these methods is about 1 m, the lower end of the 1–10 m range we construe from hand pollination to contain the optimal outcrossing distance for *D. nelsoni*. This discrepancy may indicate our inability to measure pollen flow directly or to define optimal outcrossing precisely using hand pollination. On the other hand, we do not necessarily expect to find congruence between actual pollen flow and that which is optimal from the point of view of female function. Instead, actual pollen flow can be expected to reflect: (1) conflicting selection on plants to maximise amounts of pollen received as well as to optimise transfer distances (for example, bees and birds may move more pollen than birds alone even though birds move slightly longer distances); (2) conflicting selection on plants to donate as well as receive pollen over optimal distances (for example, optimal dispersal distances may differ for male and female functions); or (3) conflicting selection on pollinators to minimise flight distances and on plants to achieve optimal outcrossing. In (3) it is difficult to see how plants can exert any major influence on pollinator flight distances by altering floral rewards. For this reason, the ability of plants to discriminate pollen genotypes after pollination[25–27] may be of major importance if they are to achieve optimal outbreeding, assuming that pollinators transfer some minimal amout of pollen an optimal distance.

Although we suggested that an intermediate optimal outcrossing distance could be caused by intragenotypic, intergenotypic, or genotype × environmental effects, our data most strongly implicate the first effect. Between-treatment differences in seedling survivorship appeared even though we planted seeds from 1976 hand pollinations in low densities far from the maternal meadow. As they occurred in the probable absence of sibling competition and in environments not similar to those of female parents, these differences suggest that physiological compatibility between parental genotypes is important in determining offspring fitness. We are now attempting to determine the importance of intergenotypic and genotype × environmental effects in molding the breeding system of *D. nelsoni*, by measuring the influence of seed dispersal distance, sibling competition, and environmental variation on the viabilities of variously outcrossed progenies.

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Bimodal distributions of seedling weight in relation to density of *Festuca paradoxa* Desv.

THE degree to which plants growing together interfere with one another by competing for resources such as light, moisture and nutrients is difficult to determine by direct measurement of resource depletion[1]. Plant ecologists have therefore looked for indicators of interference in population structure[2,3]. One proposed measure of interference is the shift that occurs from the 'normal' distribution of seed weight to a 'log-normal' distribution of the weight of seedlings grown from those seeds in an even-aged monoculture. This shift presumably reflects the emergence of a few large and dominant individuals and the suppression of larger numbers of small individuals. Recently Ford[4] and Newbould[5] have proposed that distributions of plant weight in competing populations are actually bimodal, resulting from the presence of distinct populations of large individuals with positive relative growth rates and small individuals with nearly zero growth rates. Even-aged plantations of *Picea sitchensis* demonstrate such disjunct growth rates, and mortality is confined to the smaller individuals[4]. Size distributions in naturally thinning populations of *Abies balsamea* and *Prunus pensylvanica* are also bimodal[6]. We have studied the distribution of seedling weight in even-aged stands of *Festuca paradoxa*, a native North American prairie grass[7], and report here that although the distribution is bimodal, the bimodality is more striking at lower densities, where interference is less severe. This result is the reverse of that expected if the extent of bimodality were a direct indication of the magnitude of interference. Therefore, although bimodality may be an eventual outcome of interference, it cannot be used as a measure of the strength of interaction between individuals.

At the highest density (16 seeds cm^{-2}), distributions of ln-transformed seedling weight (Fig. 1) are only slightly or not at all bimodal. The distributions become increasingly bimodal as density increases. At the lowest density (1 seed cm^{-2}), the distribution appears most strongly bimodal. All distributions are significantly different from the normal distribution at the 5% level (*G*-tests[8]). Only the distributions at 16 seeds cm^{-2} show significant skewness (to the right[9]), but all are significantly platykurtic (flat-topped[9]). Platykurtosis is not sufficient to demonstrate bimodality, but bimodal distributions are platykurtic. There is no direct statistical measure of bimodality.

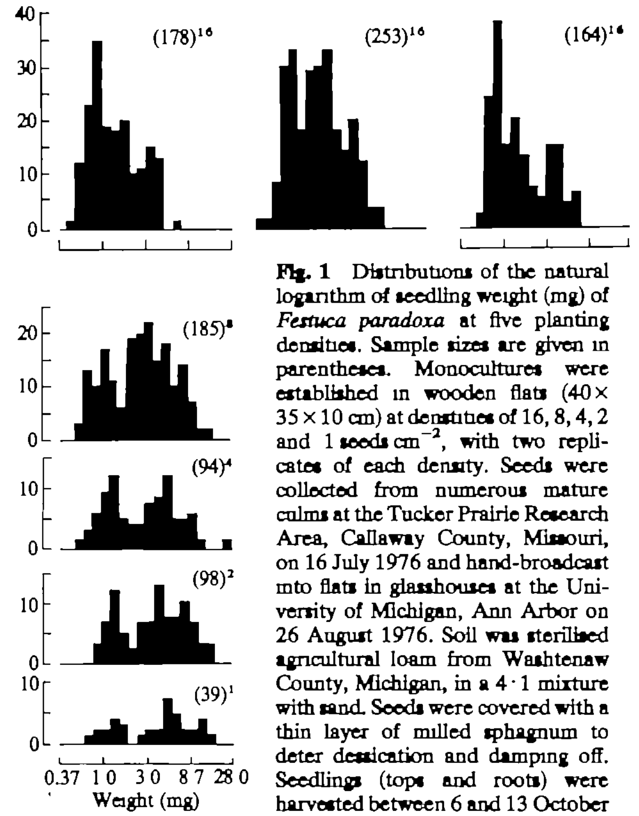

Fig. 1 Distributions of the natural logarithm of seedling weight (mg) of *Festuca paradoxa* at five planting densities. Sample sizes are given in parentheses. Monocultures were established in wooden flats (40 × 35 × 10 cm) at densities of 16, 8, 4, 2 and 1 seeds cm^{-2}, with two replicates of each density. Seeds were collected from numerous mature culms at the Tucker Prairie Research Area, Callaway County, Missouri, on 16 July 1976 and hand-broadcast into flats in glasshouses at the University of Michigan, Ann Arbor on 26 August 1976. Soil was sterilised agricultural loam from Washtenaw County, Michigan, in a 4 · 1 mixture with sand. Seeds were covered with a thin layer of milled sphagnum to deter desiccation and damping off. Seedlings (tops and roots) were harvested between 6 and 13 October 1976 in randomly-placed 5 × 5 cm plots, 3 for each density, washed to remove soil from the roots, dried at 80 °C for 24 h, and weighed on a Cahn electrobalance. For 8, 4, 2 and 1 seeds cm^{-2}, one-way anovas[8] showed no differences in seedling weight among the three replicates at the 10% level. Consequently, the replicates were lumped, and one distribution is shown. At 16 seeds cm^{-2}, the replicates differed at the 1% level ($F_s = 4.87$) and are shown separately. Distributions at 8, 4, 2 and 1 seeds cm^{-2} are all significantly different from the distributions at 16 seeds cm^{-2} (χ^2 tests[8])

The bimodality in the weight distributions is not due to an underlying bimodality of seed weight, either for the intact diaspore or for the caryopsis without accessory tissues (Fig. 2). Neither distribution deviates significantly from the normal distribution (*G*-tests[8]), and neither is skewed or kurtotic. The bimodality is not due to an underlying bimodality in germination time (Fig. 3). This distribution is significantly right-skewed and leptokurtic.

Mean seedling weight increases with decreasing density (Table 1), indicating that resource limitation is occurring at higher densities. The bimodal pattern emerges before density-dependent mortality. The ratio of seedlings harvested to seeds planted is not significantly different for the different densities (one-way anova with arcsine transform[8]). Mortality, if occurring, is uniform among the densities.

Interference is *a priori* more severe at higher densities. Our finding that bimodality is weaker at higher density suggests that at high density, the manifestations of interference in population structure are delayed and that the interaction between individuals is slowed. At the highest density, all individuals may

Table 1 Seedling weight of *Festuca paradoxa* at five planting densities

Planting density (seeds cm^{-2})	Sample size	Geometric mean of seedling weight ± s d. (mg)	Skewness g_1	Kurtosis g_2
16	178	1.40 ± 2.23	0.444*	−0.881*
16	164	1.58 ± 1.95	0.620*	−0.893*
16	253	1.73 ± 1.88	0.258†	−0.745*
8	189	2.78 ± 2.73	−0.086	−0.935*
4	94	2.82 ± 3.29	0.125	−0.946*
2	98	3.71 ± 3.38	−0.165	−1.133*
1	39	3.92 ± 3.82	−0 318	−1.032†

Skewness (g_1) and kurtosis (g_2) are calculated for the ln-transformed distributions shown in Fig. 1.
*$P < 0\ 01$; †$P < 0.05$.

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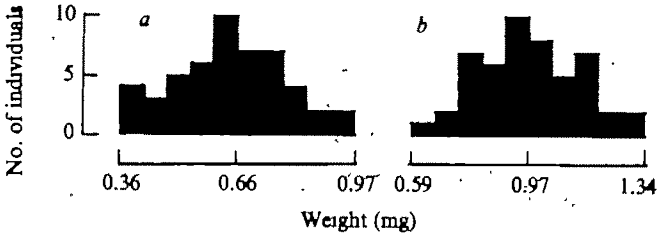

Fig. 2 Distributions of weights of excised caryopses (*a*) and intact diaspores (caryopses with lemmas and paleas) (*b*), obtained on a Cahn electrobalance. Arithmetic scale, sample size is 50 in both cases. For the intact diaspores, $\bar{x} = 0.967$ mg, $s = 0.170$, $g_1 = 0.129$ (not significantly different from zero[9]), and $g_2 = -0.576$ (NS). For the excised caryopses, $\bar{x} = 0.639$ mg, $s = 0.143$, $g_1 = 0.008$ (NS), and $g_2 = -0.502$ (NS). Neither distribution is significantly different from the normal distribution (*G*-test[8]).

suffer from more severe resource limitation; this idea is supported by the right skew in the data at 16 seeds cm^{-2}. The appearance of dominant individuals may be postponed. At lower densities, because resource limitation is less severe, growth is more rapid, individuals are larger, and the emergence of dominant individuals (and hence the bimodality) is more rapid.

Factors determining which individuals become dominant and which are suppressed remain somewhat obscure. Suppression is probably not due to genetic differences[10]. Seed size[11], order of emergence[12] and neighbourhood effects[13] have been implicated in determining which individuals become dominant.

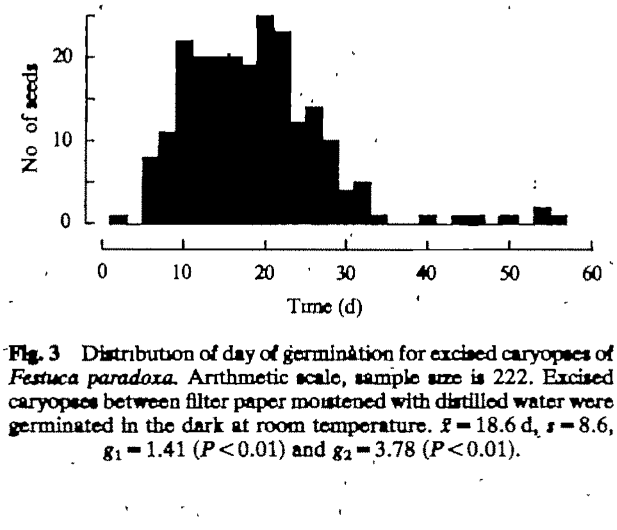

Fig. 3 Distribution of day of germination for excised caryopses of *Festuca paradoxa*. Arithmetic scale, sample size is 222. Excised caryopses between filter paper moistened with distilled water were germinated in the dark at room temperature. $\bar{x} = 18.6$ d, $s = 8.6$, $g_1 = 1.41$ ($P < 0.01$) and $g_2 = 3.78$ ($P < 0.01$).

While intrinsic factors generating bimodality cannot be exhaustively eliminated, the two most likely intrinsic sources (bimodality in seed weight or germination time) have been excluded here. The bimodality is probably a direct result of interference, although the possibility remains that severe interference prevents the expression of an intrinsic bimodality (due to other factors) at high density.

Disjunct populations with zero and positive growth rates are sufficient to generate a bimodality in distributions of ln-transformed individual weight. Bimodality appears to be an eventual structural result of interference and thus is a valuable descriptor of populational attributes. These results demonstrate the need to distinguish between populational attributes which characterise the pace and severity of interference from attributes which describe the outcome of interference.

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A method for culture of micromanipulated sheep embryos and its use to produce monozygotic twins

MICROSURGICAL techniques have proved valuable for investigations of the regulatory capacity of early mammalian embryos, including the developmental potential of single blastomeres from two-, four- and eight-celled embryos (see ref. 1 for review). However, the usefulness of radical microsurgery in experiments on early cleavage stages has been restricted largely to species in which such young embryos may be cultured *in vitro*, notably the mouse, as, in most instances, embryos less advanced than the late morula stage fail to survive *in vivo* if the zona pellucida has been removed or substantially damaged[2–6]. There has been no satisfactory method for culture *in vitro* of early cleavage stages in the sheep; and consequently there have been few reports of attempts to micromanipulate early sheep embryos[7–9]. I describe here a method whereby micromanipulated eggs can be protected during their early development *in vivo* and its use in an investigation of the developmental potential of single blastomeres from two-celled sheep embryos with the aim of producing monozygotic twins. The method involves coating the eggs with agar after microsurgery so as to seal defects produced in the zona pellucida.

In preliminary experiments it was established that young sheep embryos embedded in agar and transferred to rabbit oviducts[10] continued to develop at a normal rate, even when the zona pellucida was extensively damaged, provided that they remained embedded in agar during the culture period. On the basis of this observation, a technique was devised which enabled the production of monozygotic twins.

Two-celled embryos were collected from super-ovulated donor ewes early on day 2 of their oestrous cycle (onset of oestrus: day 0). Embryos were collected, stored, micromanipulated and transferred in an enriched phosphate-buffered saline medium (PBS)[11]. During storage and manipulation the embryos were held at room temperature.

Monozygotic pairs of agar-coated single blastomere eggs were produced as follows. (1) The two-celled egg was held by suction with a capillary pipette, and the zona pellucida was ripped open along the interblastomeric groove for about three-quarters of its circumference with a fine glass needle. (2) The blastomeres were sucked out of the zona with the aid of a Pasteur pipette with a tip diameter sufficiently small to detain the zona while allowing the

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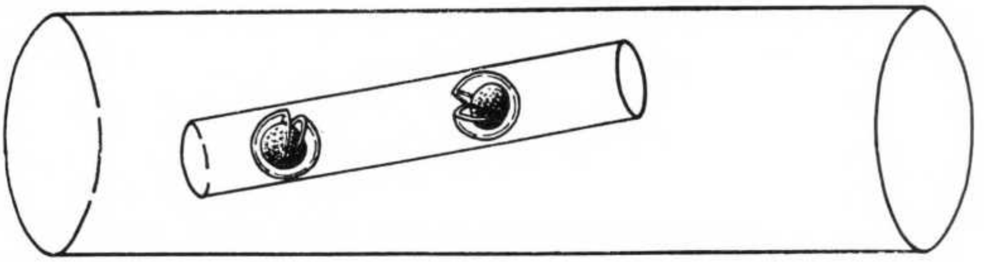

Fig. 1 Diagram of a pair of micromanipulated single blastomere eggs and agar embedding.

blastomeres to pass through. (3) The blastomeres were separated by gentle suction with a Pasteur pipette of smaller tip bore, allowing the passage of only one, slightly compressed, blastomere at a time. (4) Single blastomeres were injected with a capillary pipette into zonae evacuated as described above and held by suction. (5) A 1.2% solution of agar (Difco) in 0.9% NaCl in distilled water was prepared. While the solution was left to cool, the eggs to be embedded were transferred from PBS to sheep serum. (6) When the agar had cooled to 38–36 °C a few ml were poured into a Petri dish, and a monozygotic pair of single blastomere eggs was immediately transferred to the agar solution with a fine-bore Pasteur pipette to be picked up a few seconds later in a small amount of agar solution. The agar containing the eggs was held in the tip of the pipette for about half a minute and then expelled as a solid cylinder into PBS. The ends were cut off the cylinder so that the eggs were contained in a segment measuring 0.15×0.5–1.0 mm. (7) This tiny cylinder was in turn embedded by a similar procedure into a larger one, measuring 0.7×2.0–2.5 mm (Fig. 1). Procedures (1) and (4) were carried out with the aid of a Leitz micromanipulator and De Fonbrune suction/injection devices.

One to four agar cylinders, each containing a pair of monozygotic eggs, were transferred to each oviduct of ewes on days 1 or 2 of their oestrous cycle. To facilitate collection of eggs, the oviducts of recipient ewes were ligated at the utero-tubal junction. After $3\frac{1}{2}$ to $4\frac{1}{2}$ d the eggs were flushed from the oviducts and examined. Those embryos which had reached the late morula–early blastocyst stage were considered to have developed at a normal rate. Among the rest a distinction could be made between those which had undergone some development but were retarded and those which were completely degenerate (Fig. 2).

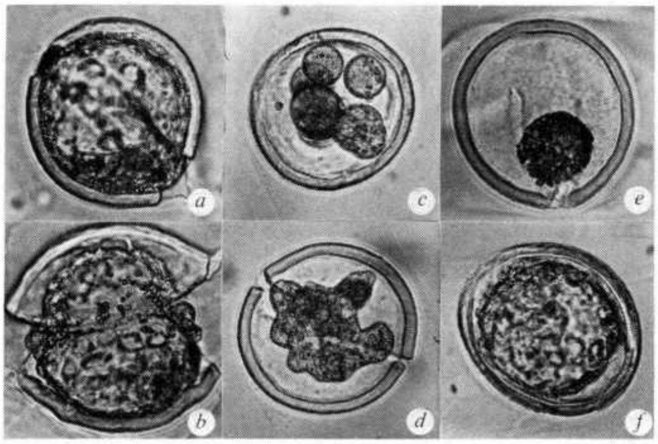

Fig. 2 Eggs from single blastomeres of two-celled sheep embryos after culture in sheep oviducts but still embedded in agar ($\sim\times400$). *a*, *b*, Monozygotic pair after $4\frac{1}{4}$ days' culture. Both eggs have developed at a normal rate and are early blastocysts. *c*, *d*, Monozygotic pair after $3\frac{1}{2}$ days' culture. *c* Is retarded in its development, whereas *d* has developed at a normal rate and undergone compaction. After fixation and staining, *c* was found to contain 8 cells, *d* 16 cells, all with morphologically normal nuclei. Normally, compaction is only evident in sheep embryos containing 30 or more cells. *e*, *f*, Monozygotic pair after $4\frac{1}{4}$ days' culture. *e* Is completely degenerate, whereas *f* is an early blastocyst. The large defects in the zonae pellucidae are visible in *a*, *b* and *d*. The main reason for inserting the single blastomeres into evacuated zonae was that the presence of the zona made it easier to remove the agar after culture, without damaging the embryo.

The results of this part of the experiment are summarised in Table 1. It is evident that very considerable losses of embryos resulted from inefficient micro- and macrosurgery on two-celled embryos and recipient ewes, respectively, but few embryos were lost in the course of the embedding procedure. Furthermore, all embryos recovered from the recipients had intact agar coatings, which made it a simple matter to recognise not only the original pairs, but also individual embryos within these pairs. The overwhelming majority of embryos recovered had also developed at an apparently normal rate. As observed in investigations of single blastomere mouse eggs[1,16], the time at which compaction and blastulation occurred reflected the age of the embryo rather than the number of cells it contained.

Table 1 Results of manipulation experiment

Pairs of monozygotic blastomeres	
Start of experiment (~no. of two-celled embryos)	61
After removal of zona pellucida, blastomere separation and injection into evacuated zonae	33
After embedding in agar	31
Transferred to ewes	31
Recovered from ewes	20
Stage of development of recovered single blastomere eggs	
Late morula or early blastocyst	35
Retarded	1
Degenerate	4
Monozygotic pairs in which both embryos had developed at a normal rate	16

Thirty-two late morulae and early blastocysts, constituting 16 sets of monozygotic embryos, were manipulated out of the agar with a pair of 24-g hypodermic needles, and transferred to ewes on days 5, 6 or 7 of their oestrous cycle. Each ewe received one pair of monozygotic embryos. Of 16 recipients, 11 became pregnant. One aborted a single fetus on day 100. The remaining 10 went to term, producing, in all, five sets of twins (Fig. 3) and five single lambs. This result represents an embryonic survival rate 10–15% below that expected following transfer of ordinary embryos on days 5–7 in sheep[12,13]. It is likely that damage resulting from the manipulations necessary to remove the agar before transplantation was responsible for the lower viability of embryos in the present experiments, and when the twinning rate is taken into consideration, it seems reasonable to conclude that each blastomere of a normal two-celled sheep embryo has the potential to develop into an entire lamb. In view of the work of Nicholas and Hall[14], Seidel[15] and Tarkowski[16] on single blastomeres of two-celled embryos of rat, rabbit and mouse, this result was not surprising, although the experiments of Mullen, Whitten and Carter[17], in which only one pair of monozygotic twins was obtained from the separated blastomeres of an unspecified number of two-cell embryos, suggested low viability of such 'half embryos'.

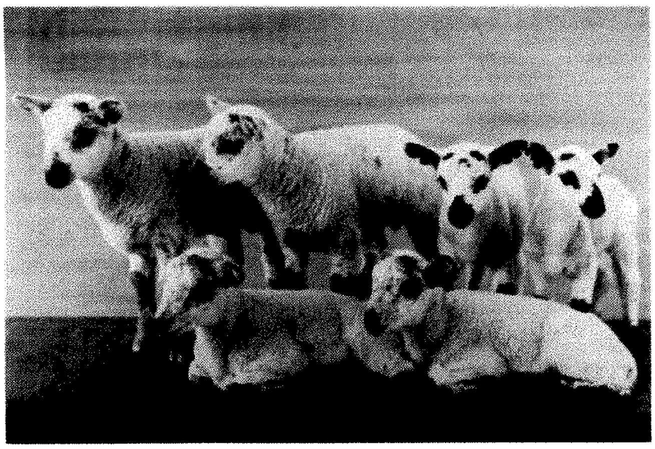

Fig. 3 Three sets of monozygotic twins produced by separation of the blastomeres of two-celled sheep embryos. Welsh Mountain cross-bred ewes, inseminated with Suffolk ram semen, were used as embryo donors. Note the general similarity in the distribution of pigmented areas, but also some minor differences between co-twins.

It may be predicted that the agar embedding method will prove useful, not only for culture *in vivo* of micromanipulated sheep eggs whether they be of the 'single blastomere' or the 'allophenic'[18] variety, but also in opening the way for similar experiments in other species, such as the pig and the cow, in which early embryos cannot yet be cultured *in vitro* with any high degree of success.

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Chromosome polymorphism and sex determination in a wild population of tsetse

SEX in the Diptera is determined by a variety of genetic mechanisms ranging from the simple condition of male or female heterogamety to complex polygenic systems[1]. Studies of tsetse flies have shown the males of all species examined to be heterogametic XY with a heteropycnotic Y chromosome[2,3]. Such cytological heterogamety is found in many dipteran species but the presence of 'sex' chromosomes does not necessarily mean that they are involved in sex determination[4]. Although there have been no studies specifically related to sex determination in *Glossina*, it has been postulated that the system is similar to that found in *Drosophila*[5]. I report here on a natural population of *Glossina palpalis palpalis* (R-D) found to contain a high incidence of aneuploids, the composition of which may be used to explain the mechanism of sex determination in the genus.

Flies were caught at five sites on tributaries of the rivers Niger and Kaduna. The sites, Akerre, Kuje, Bwari, Guni and Gimi, are located within a 100-km radius of Minna in Niger State, Nigeria. Captured females were transferred to the laboratory where they were caged individually, maintained at about 25 °C and 60–70% relative humidity and fed on rabbits. Puparia produced were collected daily and when 5 d old were used for cytogenetic analysis. Brains were dissected from the pupae, cultured for 3 h in Eagle's minimal medium containing $1\ \mu g\ ml^{-1}$ colchicine and fixed in methanol–acetic acid. Air-dried chromosome preparations[6] were made from the brain tissue and stained routinely in toluidine blue.

Production of puparia by the wild-caught flies was poor, many females producing only one puparium, the maximum obtained from a single female being five. A total of 553 puparia was obtained from 249 females, an average of 2.2 puparia per female. The normal chromosome complement of *G. p. palpalis*[3] is $2n = 4+XY$, but 26 of the females in this study (10.4%) produced offspring which were aneuploid. Of these 35 abnormal pupae, 22, which were shown to be female by dissection, were found to have XXY chromosome complements, and one was found to be XXYY; seven pupae, shown to be male by dissection, were found to be XYY (Fig. 1), and a further five pupae were X0 males. All the females producing aneuploid pupae were also shown to be capable of producing normal XX female and XY male offspring. In the case of the five X0 pupae, each came from females which produced only one such aneuploid type, the

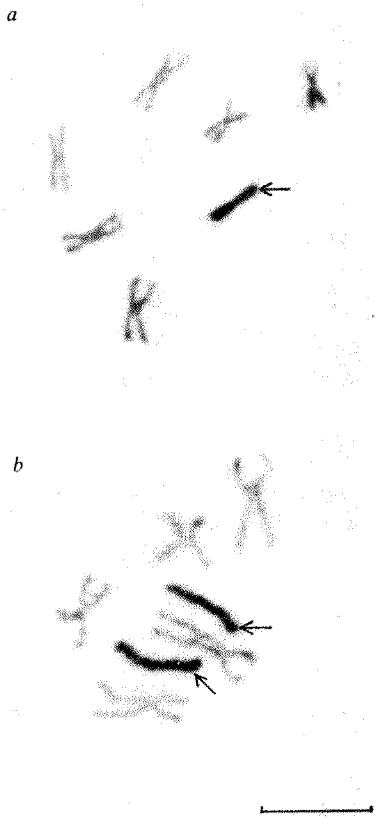

Fig. 1 Metaphase neuroblasts from 5-d-old embryos of *G. p. palpalis* showing sex chromosome aneuploidy. Arrows indicate Y chromosomes. *a*, Female, $2n = 4+XXY$; *b*, male, $2n = 4+XYY$. Bar = 10 μm.

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rest of their offspring (average three puparia per female) being normal. These figures represent the pooled data from the five collecting sites, as no significant differences were found in the incidence of aneuploidy between sites.

The presence of an extra Y chromosome, or even two Y chromosomes, in female *G. p. palpalis*, and the absence of a Y in males were found to have no obvious phenotypic effect; thus, it may be concluded that sex in this species is determined by the number of X chromosomes present. This system of sex determination is clearly analogous to that demonstrated in *Drosophila* by Bridges' classical experiments with mutant strains[4]. It was later established that sex in *Drosophila* is determined by a system of genic balance between sex determinants on the autosomes and the X chromosome[7]. Such systems are common in insects[1] and it seems probable that the autosomes play a part in sex determination in *Glossina*; however, until useful genetic markers are available, this cannot be confirmed. The X0 male pupae found in the present study all had apparently normally developing testes, but the course of spermatogenesis could not be followed in pupae of this age. Such X0 males are completely sterile in *Drosophila* due to failures at spermiogenesis[8], but until greater numbers of X0 males are obtained, the precise role of the Y chromosome in *Glossina* remains uncertain, although it has been suggested that it may not be involved in sperm development[9].

The production of sex chromosome aneuploids in this population of *G. p. palpalis* can be accounted for by non-disjunction of the X chromosome during female meiosis, giving XX and 0 eggs, the fertilisation of which by normal sperm would give rise to the XXY females and X0 males found; XXY females when crossed with normal males can be expected to produce XYY males[4]. However, the frequency of spontaneous primary non-disjunction in female *D. melanogaster* is known to be very low (0.05–0.1%)[10], and, although such data are not available for *Glossina*, the occurrence of 21 females (8.4%) in the present sample shown to be XXY (by producing XXY, XYY, XX and XY pupae) suggests that they are being maintained in the population as a polymorphism rather than by spontaneous non-disjunction. As *G. p. palpalis* is an important vector of sleeping sickness in West Africa, it may be of practical value to determine what selection pressures, if any, are maintaining this polymorphism in the population, as the epidemiology of the disease is intimately associated with the ecology of the fly[11].

If, as suggested here, sex determination in *Glossina* is controlled by a polygenic system, then it is probable that sex ratio-distorting factors are present in the tsetse genome. Such factors are commonly found in other Diptera, including mosquitoes[12] and house flies[13], which are known to have complex sex-determining mechanisms; indeed, it has been suggested that segregation distortion may be a means whereby mutant sex determinants become established in a population[14]. Sex-ratio distortion has been induced in *G. morsitans* by irradiation[5] and, in view of the present work, it would seem expedient to screen natural populations for distorting factors, as any mechanism which would increase the production of males would be most useful to tsetse control programmes which involve the release of sterile males[15].

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Giardia excystation can be induced *in vitro* in acidic solutions

MANY vertebrates, including humans, are hosts for the intestinal protozoan parasite, *Giardia*. This genus has a worldwide distribution[1] and in recent years has become an increasingly important public health problem[2–14]. Human infections may be associated with intestinal discomfort, diarrhoea, and malabsorption syndromes[15–17]. The life cycle of *Giardia* is divided into trophozoite and cyst stages. When the cyst is ingested by the host, excystation occurs (trophozoites emerge) and the upper small intestine is colonised. Although research on *Giardia* excystation began nearly 50 yr ago[18,19], conditions for the induction of the process were never defined. We demonstrate here (1) *Giardia* excystation in acidic solutions, (2) the pattern of trophozoite emergence during excystation, (3) a method for routine *in vitro* induction of excystation, and (4) the establishment of *in vitro* axenic cultures from excysted trophozoites.

Cyst-bearing faeces were obtained from humans and animals, purified by a modification of previous procedures[20,21], and stored in water at 8 °C. We attempted to duplicate the conditions to which cysts are exposed *in vivo*. Cysts were exposed to a synthetic gastric juice, transferred into growth medium and examined microscopically. Within 5 min trophozoites were seen excysting (Fig. 1). The escape of the trophozoites resembled the extrusion of a fluid-filled balloon through a small hole in one end of the cyst.

Table 1 Excystation of *Giardia* cysts exposed to inorganic acids at *p*H 2.0

Acid	Mean % excystation ± s.e.m.
HCl	17.4 ± 2.2
HNO_3	12.5 ± 3.0
$HClO_4$	15.5 ± 2.0
H_2SO_4	15 5 ± 2.6
H_3PO_4	19.4 ± 2.8
Water (control, *p*H 6.8)	0.2 ± 0.2

The mean percentage of excystation is the average of 11 trials; an average total of 2,242 cysts were counted in each acid. Mean % excystation for water differs significantly from those of all acids ($P < 0.01$); mean % excystation values for the acids are not significantly different from each other ($P > 0.01$).

To identify the factor(s) responsible for the induction of excystation, complete and component-varied synthetic gastric juice at *p*H 1.6, HSP-3 (see Fig. 1), and water were tested for ability to induce excystation. To count cysts and assess levels of excystation, the following standard procedure was developed. One volume of purified cyst suspension was added to at least 10 volumes of excystation solution (such as synthetic gastric juice, HCl(aq), water), and the mixture incubated at 37 °C for 1 h. The suspension was then centrifuged at 600*g* for 5 min at room temperature, and the pellet resuspended in water and recentrifuged. The pellet was resuspended in 0.5 ml of HSP-3 at 37 °C, and a depression slide chamber filled with the suspension, sealed with a cover glass and paraffin–Vaseline, and incubated

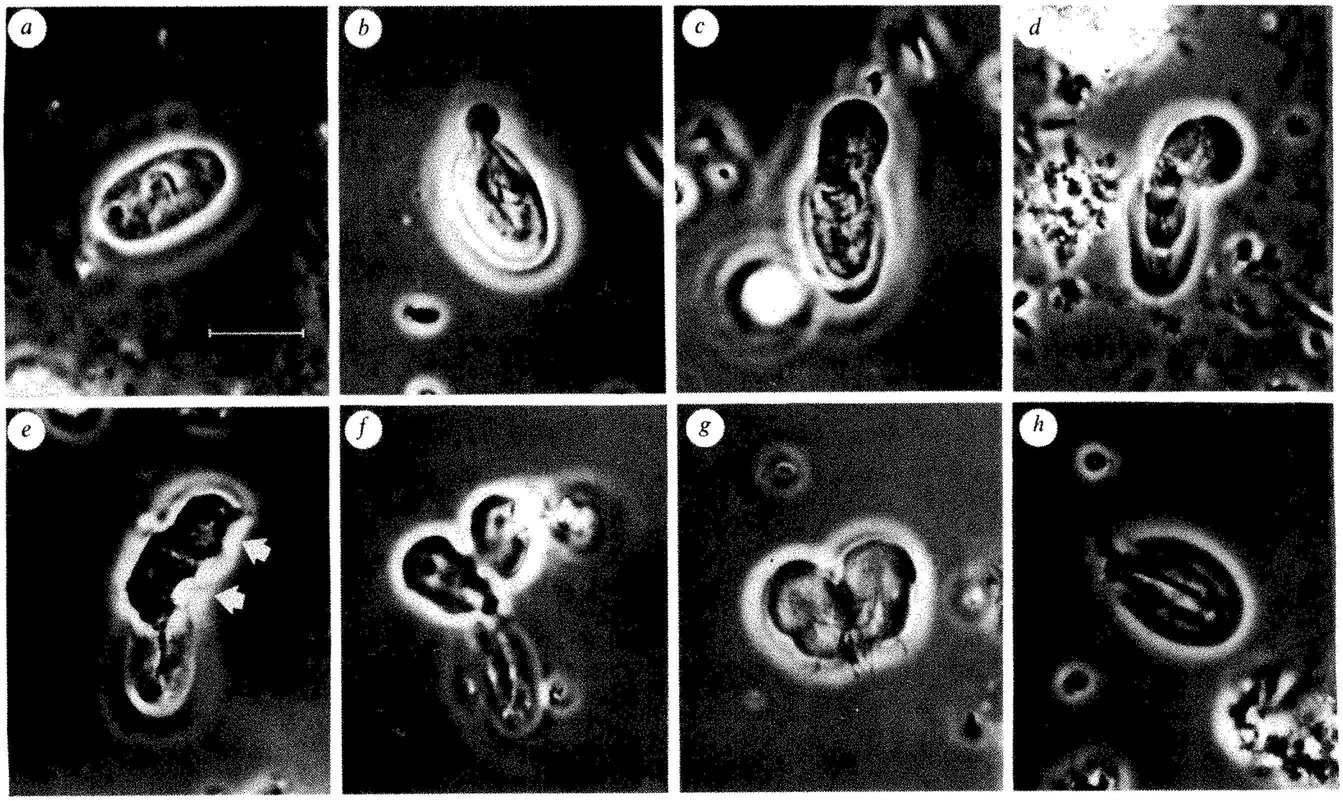

Fig. 1 Representative steps in *Giardia* excystation. *a*, Typical cyst; *b–f*, sequential emergence of trophozoite (arrows in *e* indicate ventral adhesive disks of daughter trophozoites); *g*, excystation completed, division of daughter trophozoites continuing; *h*, empty cyst. The entire process normally requires 5–30 min. Cysts were exposed to 37 °C for 2 h to a synthetic gastric juice containing the following components in aqueous solution: $NaHCO_3$ (25 mN), KCl (12 mN), NaCl (40 mN), $CaCl_2$ (12 mN), and pepsin (1,500 units ml^{-1}; Sigma); final *p*H 1.6 (refs 22, 23). Following exposure to the synthetic fluid, the cysts were transferred into 37 °C HSP-3, a *Giardia* growth medium derived from Meyer's HSP-1 and HSP-2 media[24] with the following formulation: 85 ml Hanks–phytone broth, 20 ml Seitz filter-sterilised heat-inactivated human serum, 7.5 ml NCTC-135 (Gibco), 1.5 ml 1.0 M $NaHCO_3$, 200,000 units potassium penicillin G, 0.01 g streptomycin sulphate and 0.01 g gentamicin sulphate; final *p*H 7.0. Photographs were taken under phase contrast at ×630 on a Zeiss Invertoscope D with Kodak SO-115 film. Scale bar, 10 μm.

inverted at 37 °C for 1 h. The cover glass was scanned systematically at ×300 on an inverted microscope, and cysts and trophozoites were counted. It was found that exposure of cysts to water and HSP-3 yielded negligible ($<0.1\%$) excystation, whereas the other solutions induced 22–26% excystation. Salts and pepsin did not significantly alter the level of excystation in these solutions (Student's *t* test, $P>0.01$), indicating that HCl alone was capable of inducing the process.

Subsequent experiments with HCl(aq) at *p*H values between 0.5 and 6.8 revealed peak percentages of excystation between *p*H 1.3 and 2.7, with a significant drop in excystation above and below peak values ($P<0.01$), and progressively diminishing excystation as the *p*H approached neutrality. These results thus demonstrate a relationship between *p*H and levels of excystation. To determine whether the factor inducing excystation was hydrogen ion, chloride ion, or a combination of the two, several acids were adjusted to *p*H 2.0 and excystation attempted by the standard procedure. Each acid induced essentially identical levels of excystation (Table 1), indicating that the hydrogen ion induced excystation regardless of the counterion.

Giardia excystation therefore seems to be a *p*H-dependent process initiated by acidic conditions encountered during gastric passage. Using the standard excystation procedure with *p*H 2.0 HCl, we have induced excystation of *Giardia* from humans, monkeys, dogs, rats and mice, and have established cultures from excysted trophozoites of cysts obtained from humans and monkeys. These cultures have been maintained for up to 7 months in HSP-3, and most were cultivated axenically from the time of excystation.

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Arrested proliferation of radial glial cells during midgestation in rhesus monkey

AUTORADIOGRAPHIC analysis using ^{3}H-thymidine labelled DNA demonstrates that some radial glial cells in the fetal monkey cerebrum stop dividing during midgestation. After two months they re-enter the mitotic cycle and become transformed into astrocytes. In their amitotic period, radial glial cells remain stretched across the width of the rapidly expanding telencephalic wall. This is significant because elongated glial fibres may have a role in guidance of neuronal migration[1–3]. The pause in proliferation of radial glial cells could ensure a faithful mapping of the proliferative ventricular surface upon the expanding and convoluted cerebral cortex of primates, including man[3]. Elongated radial glial cells span the distance between the ventricular and pial surface of the developing telencephalon (Fig. 1). Studies using Golgi silver impregnations[4–7], electron microscopy[1,8,9] and immunohistochemistry[10–12] substantiate the glial nature of this transient cell class (for review see refs 12, 13). In the monkey occipital lobe, radial glial cells are present throughout the last two-thirds of gestation[12,14]. The process of transformation of radial glial cells into astrocytes and ependymal cells begins during the first half of gestation and is completed by the second postnatal month[12,14]. However, during midgestation, when neuronal migration is at a peak[15], many radial glial cells remain attached to the ventricular and pial surfaces (Fig. 1); they increase in length, and curve with the expansion and convolution of the cerebral wall[3,12,14]. The present study was undertaken to determine whether such glial cells undergo cell division after attaining their radial configuration.

Brains from 53 monkey fetuses were exposed to a single pulse of ^{3}H-thymidine (^{3}H-TdR) at various stages of gestation as part of study of primate neurogenesis (Table 1)[14–16]. Some fetuses were killed 1 h after injection (column 2, Table 1) to identify the population of actively dividing cells. Others injected at comparable gestational ages were killed at 3,7,14 or 50–60 days later (columns 3,4,5,6 respectively) to determine which cell classes continue to divide (and thereby dilute ^{3}H-TdR) as well as to show any changes in position of non-dividing cells after injection. Finally, some animals were killed several months after birth (column 7). Following fixation, one cerebral hemisphere from each animal was processed for autoradiography[16] and the other was impregnated with the rapid Golgi method[2].

Two populations of cells labelled with ^{3}H-TdR were identified on the basis of their postmitotic behaviour. In monkeys injected at E87 (embryonic day 87) or at E90 and killed 1 h after injection, all heavily labelled cells are found in the ventricular and subventricular zones, the two proliferative areas lining the lateral ventricles (Fig. 2*a*). However, in specimens injected at approximately the same fetal ages, but killed 3 or 7 days after injection, many heavily labelled cells are found in the intermediate zone and in the cortical plate while a certain number of heavily labelled cells remain in the proliferative zones. In animals killed 14 days after injections at E85 or E89, most of the migrating labelled cells have already reached their position in the superficial layers of the cortex[14]; a certain number of labelled cells are, however, still found near the ventricle at this time. In two additional fetuses injected at E85 and E90, but killed after approximately 2 months (E139 and E140, respectively), all migrating cells generated at E85 or E90 have reached their final destinations as neither the intermediate zone nor the deep cortical layers contain labelled cells. The shift in location of heavily labelled cells reflects the well documented pattern of neuronal migration to the neocortex[15]. Again, in these same cases, however, a substantial number of heavily labelled cells persist in the outer part of the subventricular zone and at the ventricular surface. The number of these cells is particularly high in areas underlying large cerebral convolutions where they form wedge-shaped aggregates (Fig. 2*b*).

These observations indicate that during the 2-month interval between ^{3}H-TdR injection and death, a certain number of cells in the subventricular zone do not divide. These non-dividing cells are unlikely to be neurones which in this region complete their proliferation by E100 and reach their final destination before E120 (refs 14, 15). The large pale nuclei of the labelled cells (Fig., 2*c*) have characteristics ascribed to radial glial cells by light and electron microscopy[1]. Furthermore, their location and pattern of distribution in the subventricular zone correspond to the dense concentration of radial glial somas revealed by the Golgi silver impregnation method in the opposite cerebral hemispheres of the same E138–E140 fetuses (Fig. 1*b*).

In specimens injected with ^{3}H-TdR at E140 and killed 1 h later, a substantial number of large pale nuclei of radial glial cells

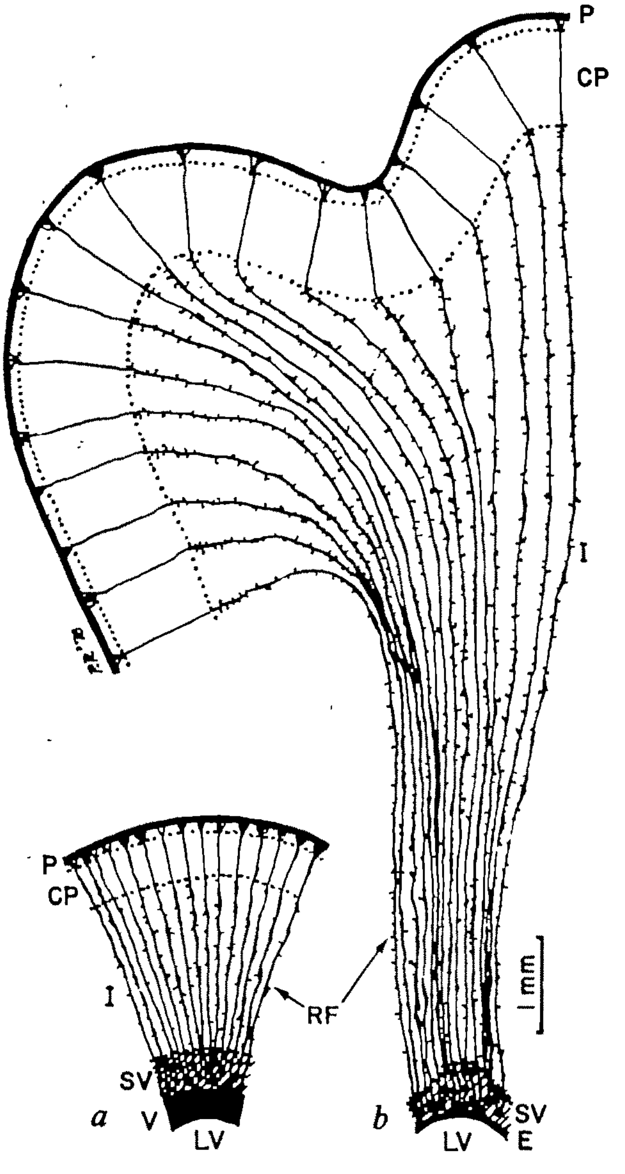

Fig. 1 Semi-schematic drawing of the distribution of radial glial cells in the telencephalon of two monkey fetuses stained with Golgi method. *a*, Dorsal portion of the occipital lobe of 81-d-old fetus (E81); *b*, section from the corresponding region from a fetus at E138. Due to the increased thickness of the cerebral wall and expansion of cortical plate during the intervening period, radial glial fibres that traverse the cerebral wall from the subventricular zone near the lateral ventricle to the pial surface become several times longer and considerably curved. Abbreviations: CP, cortical plate; E, ependyma; I, intermediate zone; LV, lateral ventricle; P, pial membrane, RF, radial fibre; SV, subventricular zone; V, ventricular zone. For more details see refs 1, 12 and 14.

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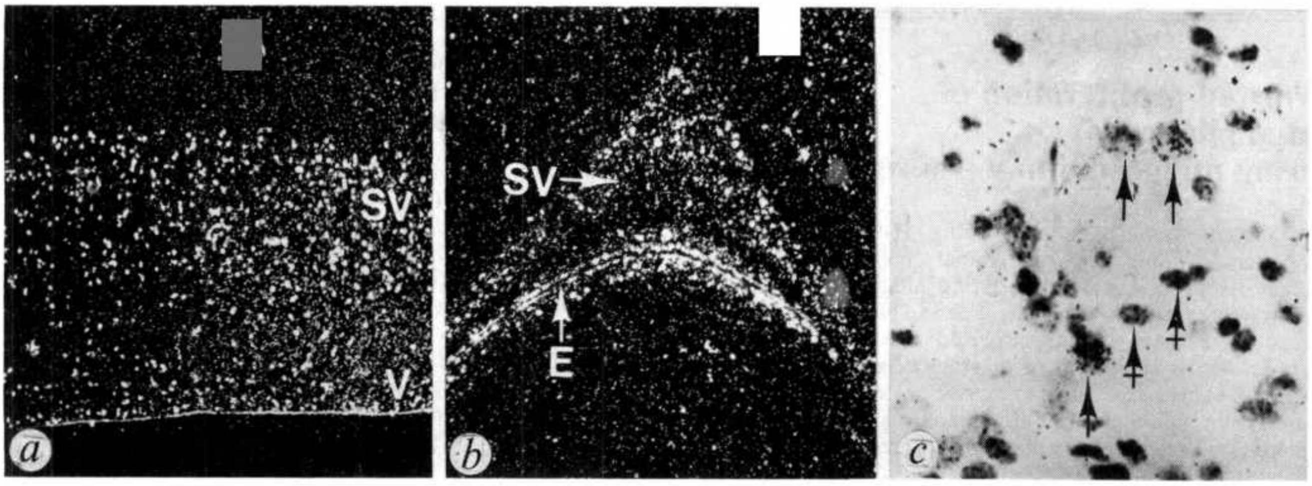

Fig. 2 *a*, Low magnification dark-field illumination photograph of the ventricular (V) and subventricular (SV) zones of the occipital lobe of a monkey fetus injected with ^{3}H-thymidine at embryonic day 90 and killed 1 h later. The confluence of silver gains over radioactive nuclei is such that labelled cells at this magnification appear as solid bright dots. Labelled cells are present in both proliferative zones. *b*, Similar photograph of the corresponding area in monkey fetus injected at E90 but killed at E140. By this fetal age the ventricular zone is exhausted and is replaced with the ependymal layer (E). Although during the 50-day period between injection and death most labelled cells have migrated to the cortex, many heavily labelled cells are still present in the subventricular zone. In the areas subjacent to large convolutions like the one selected here, aggregates of labelled cells form wedges that correspond to the accumulation of radial glial cell somas in Golgi impregnation (for example, see Fig. 1*b*). *c*, Bright-field photograph of large, pale, labelled nuclei (arrows) and unlabelled dark nuclei (crossed arrows) in the subventricular zone of a monkey fetus injected at E85 and killed at E139. The dark unlabelled nuclei apparently represent regular proliferating population of subventricular cells which incorporate ^{3}H-thymidine throughout gestation, but never retain label over long periods.

are labelled in the subventricular zone. However, in monkeys injected at either E90 or E140 but killed postnatally, no labelled cells can be detected in this general region of the occipital lobe except for an occasional ependymal cell. This finding suggests that a certain number of radial glial cells, which have not divided for almost 2 months, re-entered the mitotic cycle around E140 and thereafter divided a sufficient number of times to dilute label beyond detection by autoradiographic method.

Not all radial glial cells in the monkey pass through this amitotic period. It seems that the majority of cells that enter this dormant phase are generated during midgestation. This explains why in specimens killed 14 days after a ^{3}H-TdR injection given earlier or later than the midgestational period (Table 1) only occasional cells in the subventricular zone remain heavily labelled. Evidently, relatively few radial glial cells generated at those fetal ages become non-dividing. Why some radial glial cells enter a mitotically dormant stage, and what induces them to re-enter the mitotic cycle remain to be determined. It is noteworthy that radial glial cells in the amphibian optic tectum can be induced to re-enter their mitotic cycles by transection of the optic nerve[17]. Thus, it is possible that the interaction of the radial fibre with the neuropil below the pial surface may mediate the proliferative activity of glial cell somas situated near the ventricular surface.

The non-dividing cell population in the primate subventricular zone is formed during the period of rapid expansion of the cerebral wall and formation of the cerebral convolutions. The 2-month dormant period during midgestation would explain how some radial glial cells maintain stable positions at both surfaces of the cerebral wall during this period (Fig. 1*a*, *b*). Their amitotic state may be correlated with their proposed function as guides for migration of late-generated neurones[1] and may assure columnar grouping in the cortex of neurones generated at common sites in the proliferative zones[3].

Table 1 Specimens used for the determination of proliferation of glial cells at various stages of development

	Survival after ^{3}H-thymidine injection					
(1) Age-matched grouping	(2) 1 h	(3) 3 days	(4) 7 days	(5) 14 days	(6) 1–2 months	(7) Killed after birth
E40–43	E41–E41.1	E41–E44	E40–E47	E40–E54	E41–E83	E40–P62
E44–48	E45–E45.1	E45–E48	E46–E53	E45–E59	E45–E78	E45–P58
49–53	E50–E50.1	—	—	E50–E64	—	E50–P61
E54–60	E58–E58.1	—	—	E56–E70	—	E56–P66
E61–67	E62–E62.1	—	—	E62–E76	—	E62–P50
E68–74	E69–E69.1	—	—	E70–E84	—	E70–P98
E75–81	E75–E75.1	—	—	E77–E91	—	E80–P48
E82–88	E87–E87.1	E82–E85	E85–E92	E85–E99	E85–E139	E85–P94
E88–97	E90–E90.1	E92–E95	E90–E97	E90–E104	E90–E140	E90–P63
E98–108	E105–E105.1	—	—	E100–E114	—	E102–P65
E109–117	E110–E110.1	—	—	E110–E124	—	E110–P62
E118–130	E120–E120.1		—	E120–E134	—	E120–P113
E131–149	E140–E140.1	E135–E138	E142–E149	E135–E149	—	E140–P57

Column[1] (age-matched groupings) refers to the time period during which a single intravenous injection of ^{3}H-thymidine (H-TdR) was administered to the pregnant monkey. Gestation in rhesus monkey lasts about 165 days. Animals are killed at various intervals after injection, as indicated by the column headings(2–7). All specimens in any one horizontal row make up one age-matched grouping. E = Embryonic day, P = postnatal day. Each hyphenated set of ages in columns 2–7 represents a single monkey, indicating first the age at time of ^{3}H-TdR injection and then the age at the time of death. The numeral 1 in the 2nd column (for example, E58–58.1) indicates survival time of 1 h following injection at a given embryonic day.

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Production of an H-2-related suppressor factor by a hybrid T-cell line

CELL FUSION has been used to study problems in genetics, neoplasia and virology[1]. Its application to immunology was initiated by the experiments of Köhler and Milstein[2], in which it was shown that a myeloma cell (a neoplastic B cell) could be fused with a normal antibody-producing B cell, and that the hybrids continued to secrete the specific antibody of the latter and grew as permanent lines. This type of fusion has since been used to produce homogeneous antibodies to a variety of antigens[3,4]. The same principle can also be applied to T cells, with the aim of producing T-hybrid lines with characteristic T-cell functions, such as cytotoxicity or the ability to help or suppress the responses of other lymphocytes. Recently, fusion of T cells with T-lymphoma cells has been described, leading to the establishment of lines carrying markers characteristic of the normal T-cell partner[5–7], and some lines with T-cell activities have been reported[8,9]. We describe here the production of a hybrid line which exhibits a characteristic T-cell function, namely, specific suppression of the antibody response. The hybrid cells produce a suppressor molecule or 'factor' which binds to an antigen, sheep red blood cells (SRBC), and specifically suppresses the antibody response to this antigen *in vitro*. The factor is non-immunoglobulin, but carries determinants of the H-2 complex.

Hybridisation was carried out using the thymoma line BW 5147 (derived by Dr R. Hyman), which lacks the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) and consequently can be killed by medium containing HAT (hypoxanthine, aminopterin, thymidine)[10]; 5×10^7 thymoma cells were fused with 2×10^8 spleen cells of C57BL/10ScSn mice which had been immunised 5 d previously by injection of 0.1 ml 10% SRBC. Polyethylene glycol was used as fusing agent, after the method of Pontecorvo[11] as modified by Goldsby *et al.*[5]. After fusion, cells were seeded at about 10^6 per well in 0.2-ml volumes in 96-well culture plates (Falcon) and cultured in selective medium, that is, RPMI 1640 supplemented with 15% fetal calf serum and HAT. After 3 weeks, 58 of 214 wells seeded showed positive growth, indicating that they contained hybrid (HGPRT-positive) cells; their contents were transferred in 1-ml volumes to 24-well culture plates (Linbro) for continued growth. Further cloning of these cells was not carried out. Karyotypic analysis of several lines showed that the average chromosome number was between the normal mouse diploid (40) and tetraploid, and was in all cases higher than that for BW 5147, which has a chromosome number close to 40.

Supernatants of growing hybrid lines were tested for their ability to suppress primary antibody responses *in vitro* of either mouse spleen cells or mouse thoracic duct lymphocytes (TDL) against SRBC and other antigens. Although most supernatants, including that of BW 5147 cells, failed to influence the antibody response, a striking suppressive effect was obtained with that of a line designated A1. Table 1 shows that the A1 supernatant produced suppression of about 90% of the primary (IgM) response of spleen cells to SRBC but did not suppress the response to donkey red blood cells (DRBC). Moreover, the suppressive effect could be abolished by absorption of the A1 supernatant with SRBC, not with red cells of other species.

Using mouse TDL in a microculture system[12], the supernatant produced complete suppression of the response to SRBC and could be titrated to an endpoint of 50% suppression at a dilution

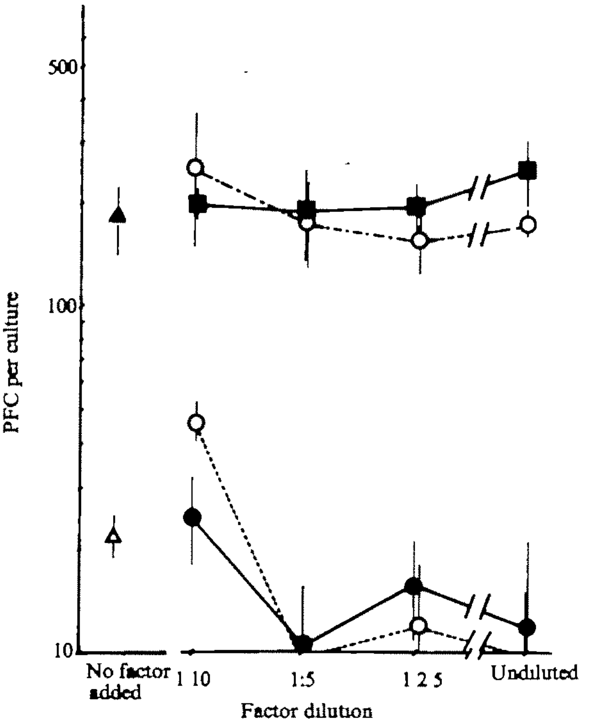

Fig. 1 Suppression of the antibody response to SRBC *in vitro* by supernatant factor of the A1 hybrid line. The figure shows the primary (IgM) antibody response to SRBC of mouse thoracic duct lymphocytes (TDL) in the presence of supernatant (factor) of A1 (●——●), A1 supernatant after absorption by SRBC (○-·-·-○) or by DRBC (○----○), or supernatant of another line, A3, as control (■——■). The level of response in the absence of added supernatant is shown (▲), as is the background in the absence of SRBC (△). The method will be described in detail elsewhere[12] In brief, 0.5×10^6 TDL of C57BL/10ScSn mice (an overnight collection of mouse thoracic duct cannulated according to ref 20) were incubated with 1×10^6 SRBC and 25 µl of the factor to be tested (or a dilution thereof) in a total culture volume of 225 µl, in round-bottomed microtitre trays (Linbro IS-MRC-96TC) under 5% CO_2/air at 37 °C. The culture medium was RPMI 1640 supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U ml^{-1} penicillin–streptomycin and 1×10^{-5}M 2-ME. After 4 d, cells in each well were collected and direct plaque-forming cell (PFC) numbers determined[19]. Each point is the geometric mean of triplicate cultures ±s.d.

Table 1 Suppression of the primary antibody response *in vitro* by supernatant of the T-hybrid line A1

Supernatant	Primary PFC response to: SRBC	% Suppression	DRBC	% Suppression
None	3 29(1960) ± 0.05		3.06(1160) ± 0.07	
BW 5147	3.25(1760) ± 0.03	10	3.09(1240) ± 0.04	0
Line A3	3.31(2040) ± 0.06	0	3.01(1030) ± 0.05	11
Line A1	2.24(174) ± 0.13	91	2.99(986) ± 0.05	15
A1 after absorption by:				
SRBC	3.24(1720) ± 0.07	12	ND	
DRBC	2.45(280) ± 0.15	86	ND	
Ox RBC	2.62(420) ± 0.10	79	ND	
anti-mouse antiserum	2.53(338) ± 0.20	83	ND	
anti-IgM	2.19(158) ± 0.19	92	ND	
anti-IgG$_{2a}$	2.48(300) ± 0.13	85	ND	
anti-IgG$_{2b}$	2.56(360) ± 0.22	82	ND	
anti-IgA	2.31(204) ± 0.24	89	ND	
anti-H–2^k	2.28(193) ± 0.23	90	ND	
anti-H–2^b	3.33(2130) ± 0.03	0	ND	

Results are primary IgM plaque-forming cell (PFC) responses of spleen cell cultures. Spleen cells of non-immunised C57BL/10ScSn mice were suspended at a density of 6×10^6 per ml and incubated with 50 µl 1% red cells in 1-ml volumes in culture tubes (Falcon) for 5 d, as described by Fridman and Golstein[18]. The culture medium was RPMI 1640 with addition of 2 mM glutamine, 1 mM pyruvate, 100 U ml^{-1} penicillin–streptomycin, 10% fetal calf serum, 1% horse serum, 5×10^{-5} M 2- mercaptoethanol (ME), and tubes were incubated under 5% CO_2/95% air. At the start, 100 µl of hybrid supernatant, taken after 48 h incubation of 5×10^5 hybrid cells in fresh medium (RPMI 1640, 15% fetal calf serum, glutamine, pyruvate, antibiotics as above) was added. Direct PFC were enumerated on day 5 in agar-free chambers[19]. Results as PFC per 10^6 viable cells, expressed as $\log_{10}$ geometric means of quadruplicate cultures (anti-log in parentheses) ± standard deviation. For absorption by red cells, 1 ml A1 supernatant was mixed with 0.1 ml 50% red cells for 1 h at room temperature and then centrifuged. For other absorptions, supernatant was passed over immunoadsorbents prepared by conjugation of antisera to CNBr-activated Sepharose (Pharmacia). Anti-mouse antiserum was raised in sheep against whole mouse serum; the specific anti-immunoglobulin reagents were also sheep anti-mouse and were anti-MOPC 104E(IgM, λ), anti-Adj PC5 (IgG$_{2a}$, κ), anti-MOPC 141 (IgG$_{2b}$, κ) and anti-MOPC 315 (IgA, λ). Anti-H–2^k was C57Bl/10 anti-C3H; anti-H–2^b was C3H anti-C3H.B10. Line A3 was a non-suppressor line from the same hybridisation, as negative control. DRBC, donkey red blood cells. ND, not done Background (antigen omitted from culture): 1.89(79) ± 0.30 (SRBC), 1.60(40) ± 0.33 (DRBC).

Table 2 Suppression by T-hybrid line Al supernatant of the primary response of C57BL/10 thoracic duct lymphocytes to SRBC and TNP$_{10}$-SRBC *in vitro*

Antigen	Hybrid supernatant	Undiluted	Dilution of supernatant 1:2.5	1:5	1:10	1:20
SRBC	Line A3	2.32(209) ± 0 04	ND	ND	ND	ND
	Line Al	0.30(2) ± 0.32	0.38(2) ± 0 29	1.52(33) ± 0.19	1.88(76) ± 0.13	2.27(186) ± 0 15
	Medium control		2.36(230) ± 0 04			
	Background		1.13(13) ± 0.34			
TNP$_{10}$-SRBC*						
	Line A3	2.15(140) ± 0.20	ND	ND	ND	ND
	Line Al	0.50(3) ± 0.33	0.30(2) ± 0.38	1.13(13) ± 0.35	1.52(33) ± 0.18	2.12(131) ± 0.08
	Medium control		2.09(123) ± 0.04			
	Background		0.38(2) ± 0.22			

Cultures of TDL were set up as described in the legend to Fig. 1, the initial cell number being 0.5×10^6 per culture. Either SRBC or trinitrophenylated SRBC (TNP$_{10}$-SRBC, ref. 21) was added as antigen (1×10^6 per culture), together with 25 µl of hybrid supernatant or a dilution thereof. After 4 d, direct PFC were measured against SRBC or TNP$_{10}$-DRBC* Results as $\log_{10}$ geometric means PFC per culture (anti-log in parentheses) ± standard deviation. Line A3 was a non-suppressor line used as negative control. As medium control, normal medium was used instead of hybrid supernatant, and for background values, antigen was omitted from the cultures. ND, not done.

Table 3 Binding of anti-immunoglobulin and anti-H–2 antibodies to SRBC treated with supernatant of T-hybrid lines

Hybrid supernatant	Binding (c.p.m.) Anti-Fab	Anti-μ	Anti-H–2^b	Anti-H–2^k
None	73 ± 14	93 ± 20	86 ± 16	107 ± 17
Line A3	46 ± 9	81 ± 19	114 ± 21	88 ± 23
Line A1	58 ± 10	70 ± 12	701 ± 66	65 ± 11
Positive controls (anti-SRBC antiserum)		anti-Fab: 4,330 ± 263 anti-μ . 3,720 ± 186		
c.p.m. of ^{125}I-reagents		anti-Fab: 15,500 ± 294 anti-μ · 14,200 ± 318		
Machine background		35 ± 4		

100 µl of each supernatant was reacted with 25 µl 10% SRBC; cells were washed 3 times, then incubated with 25 µl of ^{125}I-labelled sheep anti-mouse Fab or sheep anti-mouse μ, or with 25 µl undiluted anti-H–2 antisera followed, after washing, by the ^{125}I-anti-Fab reagent. As positive controls SRBC were treated with mouse anti-SRBC antiserum followed after washing by the labelled anti-Fab or anti-μ. After further washing, the c.p.m. bound to red cells were counted. Anti-mouse Fab was raised in sheep against the Fab fragment of Adj PC5 (IgG$_{2a}$, κ), anti-μ was sheep anti-MOPC 104E (IgM, λ) absorbed with λ light chains; anti-H–2 antisera were as in Table 1. Results as means of duplicate tests ± standard deviation. Line A3 was a negative control supernatant; supernatants of 36 other lines also gave negative results.

of 1 : 20 (a final dilution in the culture of 1 : 180), as shown in Figs 1 and 2. Here too, absorption of the supernatant with SRBC removed its suppressive activity. However, a nonspecific suppression of TDL was evident at high concentrations of supernatant, when it was possible to suppress the responses to trinitrophenylated (TNP)–DRBC or TNP–ovalbumin as well as to SRBC (Fig. 2). Nevertheless, specificity for SRBC appeared as the supernatant was diluted beyond 1 : 2.5. Although the ability of the A1 supernatant to suppress the anti-TNP response to TNP–DRBC was clearly weak (Fig. 2), it suppressed the response to TNP in the form of TNP–SRBC as effectively as the response to SRBC itself (Table 2). Thus, suppression extended to haptens attached to SRBC and was evidently carrier specific. To date, we have found the supernatant to be equally effective on C57BL/10 (H-2^{b}), CBA (H-2^{k}) and BALB/c (H-2^{d}) TDL. The production by the A1 line of a suppressor factor with specificity for SRBC proved to be a stable characteristic of the line and could be demonstrated with supernatants obtained after passaging the cells for 7 months in culture.

The nature of the A1 suppressor molecule was explored by passing the supernatant over immunoadsorbents to which anti-mouse immunoglobulin (mIg) or mouse alloantisera had been chemically coupled. Table 1 shows that anti-mIg adsorbents were unable to remove the suppressive activity. In contrast, this activity was lost if the A1 supernatant was passed over certain anti-H-2 adsorbents; specifically, immobilised antisera against the haplotype of the primed spleen cells used in the fusion (H-2^{b}) were able to remove the A1 molecule, whereas those reacting with the haplotype of the thymoma (H-2^{k}) could not. Similarly, it was shown that the molecules in the A1 supernatant binding specifically to SRBC carried H-2 determinants and were non-Ig. Table 3 shows that SRBC treated with the A1 supernatant failed to bind radiolabelled anti-mIg reagents (anti-Fab, anti-μ), but did bind anti-H-2^{b} antisera (detected by subsequent binding of radiolabelled anti-mIg). In other experiments this reaction was shown to be specific for SRBC. These results indicate that the A1 suppressor factor is an SRBC-binding molecule which is non-Ig and carries determinants coded in some portion of the H-2 complex. The structure of this molecule was explored further after radiolabelling, as described in the accompanying paper[13].

Surface antigens of the A1 line were detected using antisera against H-2, mIg and Thy-1. A1 cells expressed the H-2 antigens of both BW 5147 and C57BL/10 parent cells (H-2^{k} and H-2^{b}) and both Thy-1 alleles (Thy-1.1 and Thy-1.2, respectively). A1 and all other T-hybrid lines failed to react with a variety of anti-mIg reagents, including radiolabelled sheep anti-mouse Fab and anti-μ, and fluoresceinated anti-whole mouse Ig. Similar results have been found by others[6,7] and suggest either that BW 5147 does not hybridise with B cells or that Ig synthesis is always repressed by BW 5147 genes. The A1 line formed rosettes with SRBC, indicating the presence of receptors for SRBC at the cell surface. However, we have found formation of rosettes with red cells of certain species, particularly bovine and sometimes sheep, to be a property of many T-hybrid lines, but not of BW 5147 itself. It is also seen with lines formed by fusion of myelomas with B cells, and in the majority of cases it is apparent that rosette formation is unrelated to the antigen specificity of the lines; its significance is not yet clear. Although many T-hybrid lines bind heterologous red cells, only A1 has so far been found to release soluble material reacting with red cells. (The phenomenon of rosette formation by hybrid lines will be published elsewhere[14].)

The results described here show that it is possible to produce by fusion a hybrid line which retains a functional characteristic of a normal T-cell population after antigen stimulation. This and similar lines[8,9] promise to be of considerable value in studying the properties of the molecules produced by normal T cells, including receptors for antigen. From the characteristics of the A1 line, we suggest that the normal parent cell in the fusion may have been an SRBC-specific suppressor T cell. Thus, the suppressor molecule it releases resembles the antigen-specific factors which have been found in cultures or extracts of antigen-stimulated T cells and which can replace T cells in regulation of the antibody response[15,16]. Although the A1 line has not yet been cloned, it is likely that the factor it produces is homogeneous and recognises a single type of determinant on SRBC. Nevertheless, it is able to suppress the anti-SRBC response completely and can also inhibit the response to a hapten coupled to SRBC. Thus, it seems unlikely that its suppressive action is due solely to blocking of antigenic determinants on the red cell surface. A direct effect on lymphocytes is indicated by the apparently nonspecific suppression which A1 supernatants produce when at sufficiently high concentration (Fig. 2) and by more recent observations we have made which indicate that the A1 factor is absorbed by B cells in the absence of SRBC[17]. Our hypothesis is that the factor reacts with both B lymphocytes and SRBC and that its greater effectiveness against the latter than against other antigens is due to its binding site for SRBC, which causes it to be concentrated at the surface of SRBC-binding B cells.

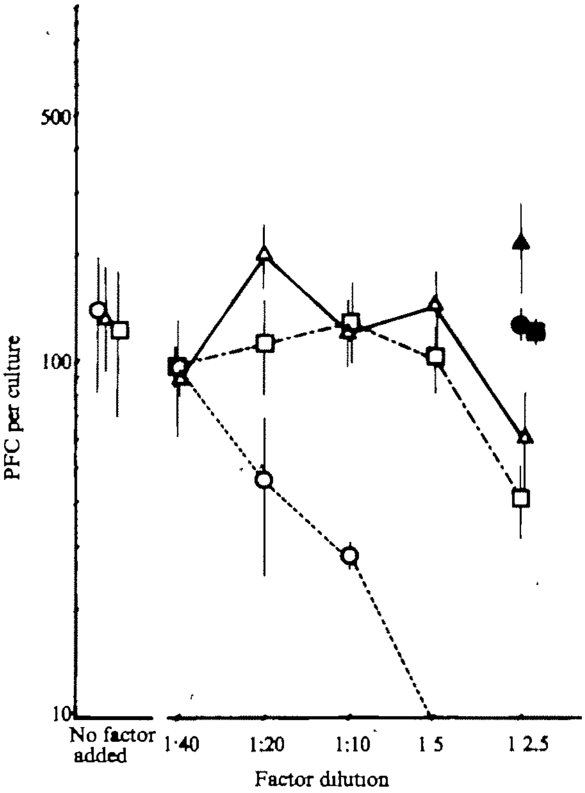

Fig. 2 Specificity of suppression of the antibody response by A1 supernatant factor. Culture conditions were as described in Fig. 1, using in this experiment TDL of CBA mice Antigens added to the cultures were 1×10^{6} SRBC (○), 1×10^{6} TNP$_{10}$–DRBC (△) or 0.125 μg TNP$_{5}$–ovalbumin (□), together with 25 μl of A1 factor or a dilution thereof (trinitrophenylation of DRBC was according to ref. 21). PFC were determined against SRBC or TNP$_{10}$–SRBC[21] after 4 d. Controls are shown in which supernatant of the A3 line was used instead of A1 (closed symbols) or in which no supernatants were added.

An important point of similarity with other specific T-cell factors is that although the A1 factor binds specifically to SRBC, it lacks Ig determinants and carries antigens coded by the H-2 complex[15,16]. Until now the main drawback to determination of the structure of antigen-specific T-cell factors has been the very small amount of material available, a problem which is overcome by the release of factors by permanent hybrid lines. In the accompanying report, some molecular characteristics of the suppressor factor produced by the A1 line are described.

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Structure of an antigen-specific suppressor factor produced by a hybrid T-cell line

THE preceding report[1] described the production by the hybrid T-cell line A1 of a suppressor factor with specificity for sheep red blood cells (SRBC). The factor, which binds specifically to SRBC and suppresses the antibody response to this antigen in culture, does not react with anti-mouse immunoglobulin (mIg) reagents, but is removed by mouse alloantisera (anti-H-2). In these respects it resembles the antigen-specific T-cell factors which have been obtained from T cells after stimulation by antigen and which play an important part in regulation of the antibody response[2,3]. We report here our study of the structure of the A1 suppressor molecule by incorporation of ^{3}H-leucine, followed by SDS–polyacrylamide-gel electrophoresis (SDS–PAGE). The molecule seems to be composed of two types of polypeptide chain in noncovalent association, a large chain of molecular weight 85,000 and a small chain of 25,000. The MW of the native molecule is about 200,000. The isolated large chain binds to SRBC. The H-2 determinants are apparently present on the small chain.

The origin of the A1 line, as a result of fusion between the AKR (H-2^k) thymoma line BW 5147 and spleen cells of a C57BL/10ScSn (H-2^b) mouse injected with SRBC, and the properties of the supernatant of the line, have been described in the preceding report[1]. The molecular weight of the A1 suppressor factor was determined by gel filtration on an ultrogel AcA 34 column (LKB), eluted fractions being tested for ability to suppress the primary response of murine thoracic duct lymphocytes against SRBC, as previously described[1]. The maximum suppressor activity emerged in the same fractions as a 7S IgM marker, indicating an apparent MW of about 200,000. No activity was found in fractions corresponding to MWs of less than 150,000 (Table 1).

Internal labelling of the A1 factor was achieved by allowing the cells to incorporate ^{3}H-leucine. Cells at a density of 2×10^6

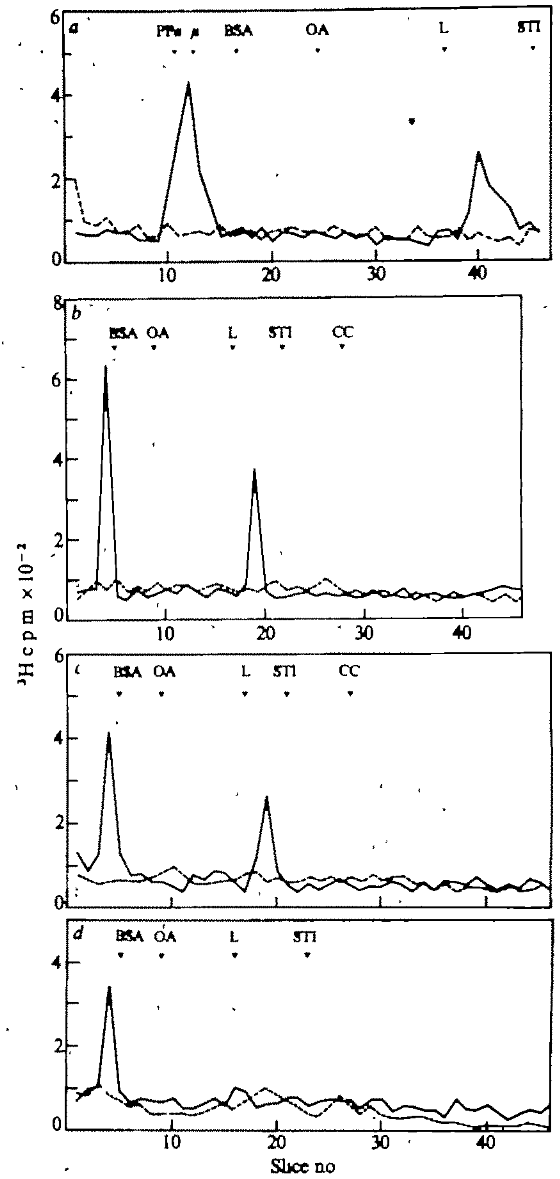

Fig. 1 SDS–PAGE profiles of SRBC-binding factor produced by the A1 hybrid line. *a*, Electrophoresis on 10% polyacrylamide gel of SRBC treated with ^{3}H-leucine-labelled supernatant of A1(——) or BW 5147(– – –). 100 µl of the labelled supernatant was incubated with 10 µl packed SRBC for 1 h at room temperature. The red cells were then washed 3 times, dissolved in SDS sample buffer in the presence of 2-ME and phenylmethanesulphonyl fluoride, heated at 100 °C for 2 min and run according to the discontinuous system of Laemmli[4]. Markers were phosphorylase *a* (PP*a*, MW 92,000), heavy (µ) and light (L) chains of MOPC 104E, bovine serum albumin (BSA, MW 68,000), ovalbumin (OA, MW 45,000), and soybean trypsin inhibitor (STI, MW 21,000). *b*, Electrophoresis on 15% polyacrylamide gel of SRBC treated with ^{3}H-leucine-labelled supernatant of A1(——) or BW 5147(– – –) Red cells prepared as in *a*. Markers as in *a* with addition of cytochrome *c* (CC, MW 12,500). *c*, Electrophoresis on 15% polyacrylamide gel of SRBC treated with ^{3}H-leucine-labelled supernatant of A1 after precipitation by anti-H–2^k(——) or anti-H–2^b(– – –) antisera and sheep anti-mouse whole serum. 100 µl of the labelled supernatant was mixed with 20 µl undiluted anti-H–2^k (C57BL/10 anti-C3H) or anti-H–2^b (C3H anti-C3H.BIO) antiserum, followed after 1 h by 250 µl sheep anti-mouse whole serum. After removal of the precipitate 10 µl packed SRBC was added to the supernatant and after 1 h at room temperature the red cells were washed and run on SDS–PAGE as in *b*. Markers as above. *d*, Electrophoresis on 15% polyacrylamide gel of fixed SRBC treated with NP40 extracts of ^{3}H-leucine-labelled A1 cells (——) or BW 5147 cells (– – –). 10 µl packed glutaraldehyde-fixed SRBC was added to 30 µl NP40 extract (see text); after 1 h at room temperature the red cells were washed and run on SDS–PAGE as in *b*. Markers as above.

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Table 1 Determination of molecular weight of A1 suppressor factor by gel filtration

Fraction no.	PFC	% Suppression
21	2 21(162) ± 0.08	0
23	2.29(195) ± 0.10	0
25	1.70(50) ± 0.18	71
27	1.02(10) ± 0.28	99
29	1.08(12) ± 0.15	97
31	1.30(20) ± 0.25	92
33	2.06(115) ± 0.11	25
35	2.20(160) ± 0.18	0
37	2.14(138) ± 0.12	9
39–61	No suppression in any fraction	
Medium control	2.18(151) ± 0.10	
Background	0.90(8) ± 0.25	

5 ml of A1 supernatant was concentrated to 0.8 ml, centrifuged to remove aggregates, and the sample applied to an ultrogel AcA 34 column (dimensions 93 cm × 2 cm^2). The column was run in phosphate-buffered saline. 3-ml fractions were collected and alternate fractions from no. 21 onwards were tested for ability to suppress the primary response to SRBC *in vitro* of mouse (C57BL/10) thoracic duct lymphocytes, as previously described[1]. Markers for MW were run separately and were shown reproducibly to emerge maximally in the following fractions: 23, blue dextran (exclusion volume); 27, 7S IgM (partial reduction product of MOPC 104E, MW 216,000); 37, μ chain of MOPC 104E (MW 80,000); 41, bovine serum albumin (MW 68,000); 44, ovalbumin (MW 44,000), 47, light (λ) chain of MOPC 104E (MW 28,000); 61, phenol red. PFC results as $\log_{10}$ PFC per culture (anti-log in parentheses) ± standard deviation. For medium control, normal medium was used instead of fraction, and for background values, SRBC were omitted from culture.

per ml were incubated for 20 h in leucine-free RPMI 1640 medium (free of fetal calf serum), supplemented with 100 μCi per ml of ^{3}H-leucine (Amersham). The labelled supernatant was allowed to react with red cells of various species and was found to bind optimally to SRBC, with some detectable binding to rabbit red cells, but none to mouse, ox or donkey (Table 2). This binding did not occur if the labelled supernatant was first treated with anti-H-2^b antiserum, followed by precipitation with sheep anti-mouse whole serum; in contrast, anti-H-2^k antiserum failed to remove the binding activity in this test, as shown in Table 1. (H–2^b is the haplotype of the primed spleen cells used in the fusion; H–2^k is that of the thymoma cells to which they were fused.) Precipitation with anti-mIg reagents, including anti-IgM (MOPC 104E) and anti-IgD (Sera Labs), or with sheep anti-mouse whole serum, also failed to remove the SRBC-binding molecule from the tritiated A1 supernatant.

SRBC which had been allowed to bind the tritiated A1 factor were washed, dissolved in SDS in reducing conditions and electrophoresed on 10% and 15% polyacrylamide gels[4] (Fig. 1*a*, *b*). This revealed two polypeptide chains, a large chain which showed an apparent MW of about 85,000 and a smaller chain of about 25,000. Similar gel profiles were obtained in the absence of 2-mercaptoethanol (ME), indicating that the chains are not linked by disulphide bonds. If the A1 supernatant was first reacted with anti-H-2^b antiserum, followed by precipitation with sheep anti-mouse whole serum, both chains were removed; reaction with anti-H-2^k antiserum and sheep anti-mouse whole serum failed to precipitate either chain (Fig. 1*c*). From these observations, it seems that the SRBC-binding molecule produced by the A1 hybrid line is composed of two types of polypeptide chain, one or both of which carries determinants coded by genes in the H–2 complex of the primed spleen cells used in the fusion.

Attempts were then made to identify the SRBC-binding molecule in lysates of the labelled A1 cells. After incorporation of ^{3}H-leucine as above, A1 cells were dissolved in the non-ionic detergent Nonidet P40 (NP40). Nuclei were spun down and the solubilised material tested first for binding to glutaraldehyde-fixed red cells (the fixation was such as to prevent lysis in NP40, but leave red cell antigens available, as evidenced by binding of anti-red cell antibodies). Table 3 shows that the NP40-extracted material from A1 bound to SRBC specifically; however, binding was not appreciably reduced by precipitation of the extracts with anti-H–2 antisera. Furthermore, when analysed by SDS–PAGE, the NP40 lysate of A1, absorbed onto fixed SRBC, showed a single peak of radioactivity, corresponding to the large chain of the A1 supernatant molecule (Fig. 1*d*). This peak persisted after precipitation of the lysate with anti-H-2^b antiserum and sheep anti-mouse whole serum. These findings imply that the H–2 determinants of the supernatant factor secreted by A1 are associated only with its smaller polypeptide chain. The absence of the latter from NP40 extracts suggests that the large and small chains may be assembled only when the molecule is secreted and that within the cytoplasm they may exist as separate pools.

Table 2 Binding to red cells of supernatant of T-hybrid line A1 after incorporation of ^{3}H-leucine

Supernatant	Treatment	Sheep	c.p.m. bound by red cells of Mouse	Ox	Rabbit	Donkey
BW 5147	—	82	71	64	63	87
A1	—	640	80	83	240	106
A1	Anti-H–2^b precipitation	166				
A1	Anti-H–2^k precipitation	519				

50 μl of supernatants of the A1 hybrid line or of the thymoma BW 5147, after labelling with ^{3}H-leucine as described in the text, were reacted with 10 μl 10% red cells of the species shown for 1 h at room temperature. The red cells were then washed 3 times and counted in a β-counter. Where indicated, A1 supernatant was precipitated, before the binding assay, by addition of 10 μl undiluted anti-H–2 antiserum to 50 μl supernatant, followed after 1 h by 150 μl sheep anti-mouse whole serum. Anti-H–2^b antiserum was C3H anti-C3H.B10; anti-H–2^k was C57BL/10 anti-C3H. Results are means of duplicate tests.

Table 3 Binding to red cells of NP40 extracts of T-hybrid line A1 after incorporation of ^{3}H-leucine

Extract	Treatment	c.p.m. bound by red cells of: Sheep	Ox	Donkey
BW 5147	—	125	85	135
A1	—	520	116	143
A1	Anti-H–2^b precipitation	405	91	76
A1	Anti-H–2^k precipitation	476	82	89

Hybrid line A1 and thymoma BW 5147 were internally labelled by growth in ^{3}H-leucine as described in the text. Extracts were prepared by dissolving 2×10^7 labelled cells in 500 μl 1% NP40 and aliquots of 25 μl were tested for binding to 50 μl 2% red cells previously fixed in 0.25% glutaraldehyde. Precipitation with anti-H–2 antisera was as in Table 2. Results are means of duplicates.

Although the structure of the A1 suppressor factor is reminiscent of 7S immunoglobulin, the chains are not disulphide linked and moreover lack the antigenic characteristics of either Ig heavy or light chains. The only antigenic determinants so far detected on the suppressor are those of the H–2 complex. The observations with labelled molecules extracted from A1 cells with NP40 indicate that the large chain is able to bind specifically to SRBC, but lacks both Ig and H–2 determinants. We conclude, therefore, that the H–2 specificities detectable on the secreted factor are probably associated with the 25,000-MW chain. As yet there is no evidence that the latter has a role in binding to SRBC.

The similarity in general properties between the A1 factor and antigen-specific T-cell factors has already been noted, in particular, the features of specific antigen binding together with H–2 association. A notable difference is the molecular weight, as the MWs of the factors obtained by sonication of suppressor T cells[3] or from T-cell supernatants[2] have been found to lie in the range of 35–55,000. It may be that the MWs of factors reported hitherto have been underestimated, perhaps due to partial proteolytic digestion before sizing.

The indication that H-2 specificities are carried by the smaller chain of the A1 factor is of particular interest. We have not yet mapped the sub-region of H-2 coding for this chain, but antigen-specific suppressor factors obtained from T cells[3] or from another T-hybrid line[5] carry Ia determinants of the I-J sub-region. The size of the small chain of the A1 factor, about 25,000, would be consistent with an origin in the I region, as mouse Ia molecules have chains of MWs about 25,000 and 35,000 (ref. 6). Although the large chain of the factor carries a binding site for SRBC, it may be the rôle of the H-2-derived chain to provide the suppressive activity of the factor. The importance of the binding site would then be to ensure a high local concentration of factor at the surface of antigen-binding lymphocytes and hence specificity of suppression[1].

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Crossreacting determinants in the C-terminal region of trypanosome variant surface antigens

ANTIGENIC variation seems to be the primary mechanism enabling the pathogenic African trypanosomes to evade the immune systems of their mammalian hosts (reviewed in refs 1–3). Antigenic variation is mediated through sequential expression of an extensive repertoire of variant surface glycoproteins (VSGs). Several VSGs have been purified from cloned variants of *Trypanosoma brucei*[4,5]. The diversity in amino acid composition, conformational features[6] and N-terminal amino acid sequences[7] implies extensive structural diversity in VSGs so far studied. Earlier studies failed to identify immunological crossreactivity between VSGs when purified or whilst on the surface of living trypanosomes. Some time ago, Dr Frans van Knapen and I, while performing enzyme-linked immunoabsorbant assays, were puzzled to observe extensive crossreactions between two purified VSGs and a wide range of antisera to different trypanosome species (unpublished experiments). A recent study by Barbet and McGuire[8] provided additional evidence for the presence of strongly crossreacting determinants on a wide range of VSGs. In several cases, the VSGs examined by Barbet and McGuire originated from clones which I previously prepared and examined. I have therefore re-examined several VSGs and their corresponding antisera by radioimmunoassay. The results reported here are consistent with those of Barbet and McGuire. Additional observations suggest that the crossreacting determinants are located towards the C-terminal end of the VSG.

As no rational basis for classification of VSGs exists, they are referred to by the stock numbers of the trypanosome clone from which they were obtained, as previously described[4]. Figure 1 shows the titration, by radioimmunoassay, of antisera of seven specificities against four variant antigens purified from randomly selected clones of *T. brucei* strain 427 (ref. 4). It is clear that VSGs 55, 221 and 121 may be substantially precipitated by certain heterologous antisera; VSG 55 (Fig. 1*a*) is most

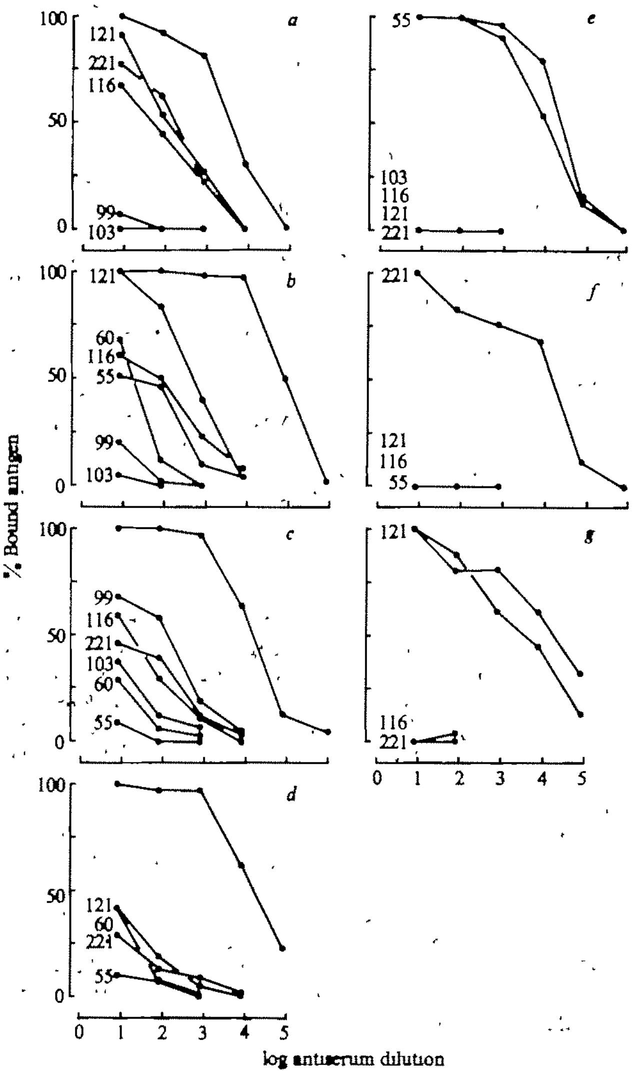

Fig. 1 Immunoprecipitation of four VSGs and three N-terminal tryptic fragments by homologous and heterologous antisera. The antigens are VSGs 55 (*a*); 221 (*b*); 121 (*c*); 116 (*d*), and N-terminal fragments from VSGs 55 (*e*); 221 (*f*); and 121 (*g*). ○, Homologous antisera; ●, heterologous antisera. Antigens were labelled by the chloramine-T method to a specific activity between 1,000 and 10,000 c.p.m. per ng. Antisera were raised in 2–4-kg New Zealand White rabbits by primary subcutaneous injection at four sites of a total of 1 mg purified VSG in an emulsion of 0.2 ml saline per 0.2 ml Freunds complete adjuvant. Two further subcutaneous injections of 0.5–1 mg VSG in saline were given at 10-d intervals. Sera were obtained 7–10 d after the third or subsequent injections of antigen and were lyophilised and stored under nitrogen at −20 °C. Radioimmunoassay was performed by incubating (2 h at 37 °C in a total volume of 0.065 ml phosphate-buffered saline[12] containing 4 mg ml^{-1} bovine albumin) 1 ng antigen with antisera dilutions prepared in appropriate dilutions of normal rabbit serum to maintain a constant amount of rabbit serum in each assay. Antigen–antibody complexes were precipitated with goat anti-rabbit IgG (Miles) and counted. Antiserum dilution is plotted against the percentage of bound antigen, after correction for nonspecific binding, taking the maximal precipitation by homologous antiserum as the 100% value. Maximal precipitation was generally equivalent to 95–100% of acid-precipitable radioactivity.

effectively precipitated by heterologous antisera 116, 121 and 221. However, an eightfold dilution was required to approach 100% precipitation. At this concentration neither antisera 99 nor 103 caused significant precipitation; 121 was the only heterologous antiserum which totally precipitated VSG 221 (Fig. 1*b*). The concentrations of heterologous antisera required to precipitate 50% of VSG 221 or 121 were 100-fold to 1,000-fold higher than the effective concentration of homologous antiserum. VSG 116 (Fig. 1*d*) was even less effectively precipitated by the four heterologous antisera tested.

The extent of heterologous precipitation demonstrated in these experiments is more variable and considerably less than that experienced by Barbet and McGuire[8]. Several factors may combine to explain both this discrepancy and my own earlier failure, and possibly that of others, to detect significant crossreactions. In agreement with our previous experiences, Barbet and McGuire were unable to demonstrate crossreactions by gel immunodiffusion or by reaction of living trypanosomes with fluorescent antisera. These were the only techniques which I routinely used for screening the antigenic specificities of trypanosomes and purified VSGs. Only minimal use had been made of radioimmunoassay procedures and these had been performed using ^{35}S-formylmethionine sulphone-labelled[9] antigens having only 1% of the specific activity of the iodinated antigens used in the current experiments. Considering the low specific activity and small range of VSGs and antisera previously used, it is not surprising that we did not detect crossreactions. Recent experience suggests that the immunisation protocol used by Barbet and McGuire, particularly the use of very low amounts of VSG, is an additional factor in obtaining strongly crossreacting antisera.

Our findings[6,10] that native VSGs may be selectively cleaved by trypsin and the resultant large and small N-terminal and C-terminal fragments separated in the absence of denaturing agents, offered the possibility of determining whether the crossreacting determinants were localised in one region of the molecule. Large undenatured N-terminal tryptic fragments of molecular weights 52,000, 41,000 and 44,000 were purified from VSGs 55, 121 and 221 respectively. Antisera were raised against the fragments from VSGs 55 and 121. The three fragments were iodinated and their precipitation by heterologous and homologous antisera was investigated.

The results given in Fig. 1 *e*, *f*, *g* show that the tryptic fragments from VSGs 55 and 121 were efficiently precipitated by homologous antisera from rabbits immunised with either the corresponding fragment or the intact VSG. The fragment from VSG 221 was efficiently precipitated by antiserum against intact VSG 221. There was no precipitation of any N-terminal fragment by heterologous antisera, in striking contrast to the results obtained with the intact VSGs. A subsequent experiment with more strongly crossreacting antisera confirmed these results. Additionally, antisera raised against the N-terminal fragments of VSGs 55 and 121 were unable to precipitate intact VSG 221. All these results strongly suggest that the crossreacting determinants are not located in N-terminal fragments representing up to 80% of the intact molecule. However, crossreacting determinants might be formed by amino acid sequences in both N-terminal and C-terminal domains being brought into proximity by the overall conformation of the intact glycoprotein. In view of the ease with which the two domains dissociate on tryptic cleavage, this seems unlikely.

Confirmation that the C-terminal fragment was the unique location of crossreactivity, was sought by inhibition studies using a glycopeptide fraction representing the C-terminal 17,000 molecular weight initial cleavage product of VSG 121 (equivalent to VSG 52 in ref. 6). Carbohydrate is found only in the C-terminal 25% of VSG 121 (refs 6, 10). Following trypsin digestion of VSG 121, a glycopeptide fraction was purified by absorption on concanavalin A–Sepharose, followed by elution and rechromatography on Sephacryl–S200. A single peak of 14,000 apparent molecular weight was recovered from the Sephacryl column. At a 10-fold molar excess, this undenatured C-terminal glycopeptide fraction was able to inhibit by 80% the immunoprecipitation of intact VSG 55 by antiserum 121 (Table 1). A 540-fold molar excess of the N-terminal 52,000 molecular weight fragment of VSG 55 did not inhibit cross precipitation: neither did a 160-fold molar excess of the N-terminal 41,000 molecular weight fragment of VSG 121. These results suggest that the crossreactions can be attributed to the C-terminal regions of VSGs 55 and 121.

The second section of Table 1 shows the competitive effects of various amounts of unlabelled intact VSGs on the precipitation of radioactive VSG 55 by a dilution of antiserum 121 which gave 60% precipitation of VSG 55 in uninhibited controls. The effectiveness of different unlabelled competitor VSGs as inhibitors varied; VSGs 55, 121 and 60 were most effective, VSGs 221 and 116 were less effective and VSG 118 at a 1,000-fold excess was only partly competitive. The reason for these variations is not apparent. One implication is that the crossreacting determinants are not shared by all VSGs. In a region the size of the approximately 150 amino acid C-terminal domain, there might be several antigenic determinants which are shared to different extents by different groups of VSGs. On the other hand, the inhibition experiments of Barbet and McGuire[8] suggest a much greater uniformity of crossreactivity between all the VSGs tested by them. This discrepancy may reflect the relatively few VSGs now being studied in either laboratory. It is possible that immunological crossreactivity will identify the existence of different VSG subgroups. The use of monoclonal antibodies[11] might clarify the situation.

The C-terminal domain of VSG 121 contains all the carbohydrate; could carbohydrate be responsible for the immunological crossreactivity? This is not easy to confirm, but three relevant observations have been made. First, concanavalin A did not block the crossreactivity between VSG 55 and antiserum 121 (Table 1). Second, VSG 118, which was the least effective inhibitor of crossreactivity, contains more carbohydrate (17%

Table 1 Inhibition of immunoprecipitation of VSG 55 by antiserum to VSG 121

Competitor (unlabelled)	ng per assay	% Inhibition of precipitation of radioactive antigen
55 f52	430	5
	43	2
121 f41	100	5
	10	0
121 f17	260	100
	26	100
	2.6	80
VSG 55	100	98
	10	74
	1	8
VSG 121	260	100
	26	100
	2.6	22
VSG 221	100	95
	10	22
	1	5
VSG 60	10	100
	1	31
VSG 116	100	95
	10	35
	1	1
VSG 118	1000	27
	100	10
	10	0
Concanavalin A	100	0
	10	0

Unlabelled competitors were diluted in PBS containing 2 mg ml^{-1} bovine albumin. Assay conditions were otherwise as described for Fig. 1 except that the total incubation volume was 0.085 ml. 55 f52 and 121 f41 are the N-terminal tryptic fragments from VSGs 55 and 121. 121 f17 is a C-terminal fragment from VSG 121.

by weight) than any other VSG studied. Third, one experiment was performed in which the 121 C-terminal precipitation-blocking glycopeptide was treated with *Streptomyces griseus* endoglycosidase H (Miles). After treatment, the C-terminal fragment was repurified by passage through a column of concanavalin A–Sepharose, on which it was no longer retained. After removal of the oligosaccharide side chain, blocking activity was not eliminated but was reduced 10-fold. This reduction could be due to conformational changes arising during the several repurification steps which were used after glycosidase treatment. The possible role of carbohydrate in crossreactivity needs further investigation.

From the evidence of its susceptibility to proteolytic cleavage[6], the VSG C-terminal polypeptide region may be rather loosely folded. We must therefore also consider that slight denaturation may expose antigenic determinants in this area which are normally masked by the native configuration of the polypeptide.

It might reasonably be assumed that VSGs have evolved by gene duplication and mutation or are made by some kind of gene-splicing mechanism. Therefore, crossreactivity is perhaps not surprising. Another argument for some conservation of structure is that all VSGs must presumably attach to the plasma membrane in a similar manner. The crossreacting determinants are apparently masked on living trypanosomes[8] and so is the C-terminal region of the VSG[10]. These facts do not greatly encourage us to believe that crossreacting determinants could provide a basis for immunisation, although this possibility will undoubtedly be explored. The significance of crossreactivity must be clarified by immunochemical and amino acid sequence studies of a range of VSGs.

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Dexamethasone-induced adhesion in hepatoma cells: the role of plasminogen activator

TRANSFORMED CELLS are frequently less adhesive than normal cells. This decrease in adhesion has been associated with the loss of specific cell surface proteins, changes in morphology, alterations in the underlying cytoskeleton and the increased activity of the serine protease, plasminogen activator[1–3]. Hepatoma tissue culture (HTC) cells exhibit increased levels of adhesion to glass[4,5] and decreased plasminogen activator activity[6,7] when incubated in the presence of the synthetic glucocorticoid, dexamethasone. Plasminogen activator converts plasminogen in serum to plasmin. It has been shown that plasmin causes the reversible dissociation of intracellular actin-containing cables in anchorage-dependent rat embryo cells[8] and may also be partially responsible for decreased adhesion of Rous sarcoma virus-transformed chicken embryo fibroblasts[3]. Cell–substrate adhesion presumably requires specific cell surface glycoproteins and intact cytoskeletal elements[9,10]. It is possible that plasmin acts either to modify 'adhesive' proteins on the cell surface or to disrupt linkages between these surface components and the underlying cytoskeleton. We have used variant HTC cells selectively resistant to the dexamethasone inhibition of plasminogen activator[6] to test the hypothesis that hormonal induction of cell adhesion is secondary to the inhibition of plasminogen activator which in turn allows the accumulation of the glycoproteins necessary for adhesion. We have modified cell adhesion assay techniques[5,11] to provide a sensitive measure of cell–substrate adhesion for both wild-type and variant HTC cells in the presence and absence of serum and plasminogen. We report here that plasminogen activator does not have an important role in the hormonal regulation of HTC cell adhesiveness.

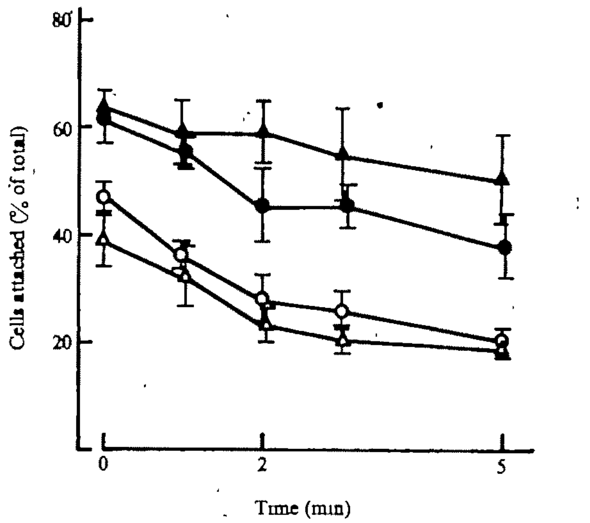

Fig. 1 Wild-type HTC cells were incubated overnight in radioactive medium containing 3% plasminogen-depleted fetal calf serum in the presence (●) or absence (○) of 0.1 μM dexamethasone, or in the same medium reconstituted with 4.8 μg ml^{-1} plasminogen in the presence (▲) or absence (△) of 0.1 μM dexamethasone. The assay conditions were the same as for cells incubated in serum-containing medium (see legend to Table 1). Plasminogen was obtained from outdated human plasma by affinity chromatography using lysine–Sepharose 4B (Pharmacia) followed by elution with ε-aminocaproic acid as described by Deutsch and Mertz[16]. Plasminogen-depleted serum was prepared by passing fetal calf serum through a lysine-Sepharose column and collecting the non-adsorbed fraction. This was acid treated to reduce the activity of protease inhibitors[17] and filter sterilised (0.45 μm pore size).

HTC cells were routinely grown in spinner culture without antibiotics in Eagle's minimal essential medium, modified to contain 2 mM glutamine, 5% each heat-inactivated fetal calf and calf serum (Grand Island Biological), 5.8 mM $NaHCO_3$ and 50 mM Tricine (Sigma). Variant HTC cell lines fully resistant to the dexamethasone inhibition of plasminogen activator were isolated using an agar–fibrin overlay technique to detect plasminogen-activator production by individual colonies of cells[6]. Glucocorticoid-receptor function was determined to be normal in variant cell lines on the basis of normal tyrosine aminotransferase induction by dexamethasone, assayed as described by Spencer and Gelehrter[12].

Wild-type HTC cells incubated for 18 h in serum-containing medium in the presence of 0.1 μM dexamethasone typically show a twofold increase in the per cent of cells remaining attached to a glass surface after 5 min of shaking, when compared to untreated cells (Table 1). After cells have been allowed to settle and attach, differences in adhesion between dexamethasone-treated and untreated cells are evident. In the presence of dexamethasone there are fewer loosely adherent cells initially, and those which remain after settling are more adherent than those in the untreated cell population. In the conditions of the assay, maximal detachment occurs within 2–5 min. We find that dexamethasone also induces adhesion in variant HTC cells which are resistant to the dexamethasone

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inhibition of plasminogen activator (Table 1). Although variant C cells are less adhesive than the wild-type HTC cells, there is a twofold increase in the adhesion of dexamethasone-treated cells incubated in the presence of serum. Two other HTC cell variants, A and B, which are also fully resistant to the dexamethasone inhibition of plasminogen activator, have similar dexamethasone-induced adhesion (data not shown). These results suggest that the mechanism by which dexamethasone increases cell-substrate adhesion does not depend on the inhibition of plasminogen activator.

When incubated in serum-free medium, wild-type HTC cells again show a twofold increase in adhesion in the presence of dexamethasone (Table 1). Variant C cells are less adhesive than the wild-type HTC cells in these conditions as well, but also show a twofold increase in adhesion when incubated in the presence of dexamethasone (Table 1). Likewise, variants A and B consistently exhibit dexamethasone-induced adhesion in the absence of serum. In both wild-type and variant HTC cells, the induction of adhesion by dexamethasone occurs to a similar extent in the presence and absence of serum.

We have also directly tested the effects of plasminogen, the substrate for plasminogen activator, on the dexamethasone induction of adhesion in wild-type and variant HTC cells in serum-free conditions. If the formation of plasmin from plasminogen prevents the accumulation of cell surface proteins required for adhesion, then the addition of plasminogen to serum-free medium should decrease or prevent dexamethasone induction of adhesion in the variant cell lines. Instead, we find that dexamethasone induces increased levels of adhesion in both wild-type and variant cells which have been incubated for 18 h in medium containing plasminogen (data not shown). This also suggests that plasminogen-activator activity does not modulate the adhesive response to dexamethasone.

Table 1 Dexamethasone induction of adhesion in HTC cells

		Per cent cells attached		
		0	2 min	5 min
+Serum (*n*)				
Wild type (4)	Dexamethasone	65±3	58±3	52±6
	Control	36±4	25±3	25±4
Variant C(4)	Dexamethasone	37±3	30±5	23±2
	Control	22±3	11±1	10±1
−Serum (*n*)				
Wild type (9)	Dexamethasone	79±1	72±2	67±3
	Control	60±5	38±4	31±4
Variant C (4)	Dexamethasone	65±5	54±6	46±6
	Control	47±10	23±6	20±4

Wild-type or variant HTC cells were collected from spinner culture during the logarithmic stage of growth. Cells were resuspended in medium containing 3% fetal calf serum, 0.1 μCi ml^{-1}, ^{3}H-amino acid mixture (NEN) and 50 μg ml^{-1} neomycin, in the presence or absence of 0.1 μM dexamethasone (Merck). After an 18-h incubation at 37 °C in a gyrotory shaker-waterbath at 80 r.p.m., the cells were centrifuged, and the cell pellet washed twice in nonradioactive medium and resuspended to a final density of 10^6 cells ml^{-1} in nonradioactive medium. Cell viability, as measured by trypan blue exclusion, was always greater than 90%. One-ml aliquots of the cell suspension were distributed into untreated borosilicate glass scintillation vials and incubated without shaking for 2 h at 37 °C. At the end of the incubation period, the cells completely covered the surface of the vial with little or no piling up of cells. The medium was removed and 1 ml calcium- and magnesium-free phosphate-buffered saline (PBS), *p*H 7.3, added carefully down the side of each vial. The vials were then subjected to a shearing force of 100 strokes mm^{-1} in a Dubnoff reciprocating shaker-waterbath (5-cm stroke) at 37 °C. At 0, 2 and 5-min exposure to this reciprocating force, duplicate vials were removed and the PBS aspirated. Cells adhering to the glass scintillation vials were lysed with 0.1 ml 1 M KOH and the lysate neutralised with 0.1 ml 1 M HCl. Radioactivity was assayed in a scintillation spectrometer after addition of 5 ml ACS scintillation fluid (Amersham–Searle). In control experiments not shown, cells were removed from the surface of vials by trypsin following the 5-min shaking period. These cells were viable by the criterion of trypan blue exclusion and the cell counts correlated with direct measurements of radioactivity in each vial. Radioactivity of the initial cell inoculum was determined from 1-ml aliquots of the cell suspension which were centrifuged, the cell pellets lysed in 0.1 ml 1 M KOH and the lysate neutralised in 0.1 ml 1 M HCl. The per cent of initial labelled cell inoculum remaining after shaking (average of duplicate vials) has been used as a measure of cell adhesiveness. The data presented represent the mean ± s.e.m. calculated from several experiments. In experiments carried out in serum-free medium (supplemented with 0.1% BSA), the assay was modified to include a 1-h incubation period for cell attachment and a reciprocating force of 140 strokes mm^{-1}.

In conditions in which plasminogen is the only serum factor missing, wild-type cells exhibit a twofold increase in adhesion in the presence of dexamethasone (Fig. 1). When cells are incubated for 18 h in medium containing depleted serum which has been reconstituted with plasminogen, a similar, although somewhat greater, increase in adhesion is measured in dexamethasone-treated cells. In the absence of dexamethasone, cell adhesion is the same whether the medium contains plasminogen-depleted or reconstituted serum. These results also suggest that plasminogen-activator production does not have a major role in cell–substrate adhesion.

Note that the optimal assay conditions differ in the presence and absence of serum. When serum is present, cell–substrate adhesion may depend on interactions between the cell surface and serum proteins which are adsorbed to the glass[13]. This might be relevant to our observation that HTC cells incubated in serum-containing medium require a longer time to form stable adhesive bonds to a substrate than do cells in serum-free medium containing bovine serum albumin (BSA). It has been proposed that cell surface proteases on BHK cells modify substrate-adsorbed proteins during the process of cell adhesion[14]. Although other cell surface proteases may be involved in cell–substrate adhesion, the nearly identical adhesiveness of untreated HTC cells in fetal calf serum, plasminogen-depleted serum and reconstituted serum provides additional evidence which tends to rule out any important role for plasminogen activator or plasmin in this process.

Recently, a class of dexamethasone-induced proteins, tentatively described as sialogylcoprotein on the basis of electrophoretic properties in a two-dimensional gel system, has been localised on the surface of HTC cells[15]. It is possible that such proteins could confer increased adhesive properties on hormone-treated cells. Our data suggest that dexamethasone regulation of cell adhesion in HTC cells probably operates primarily through the synthesis of surface glycoproteins and that this process is not, in turn, regulated by the inhibition of plasminogen-activator activity.

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Glucocorticoids regulate the concentration of 1,25-dihydroxycholecalciferol receptors in bone

THE results of a study of high-affinity glucocorticoid and 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) binding in cytosol from fetal rat calvaria cultured for up to 48 h at 37 °C are presented. The binding of glucocorticoids was found to be stable in short-term culture, whereas that for $1,25(OH)_2D_3$ declined rapidly, was almost undetectable at 24 h and had disappeared by 48 h. Furthermore, low concentrations of cortisol (10^{-8} to 10^{-6} M) added to the culture medium had a dramatic effect on the persistence of high affinity $1,25(OH)_2D_3$ binding. In the presence of cortisol there was dose-dependent preservation of most of the binding of $1,25(OH)_2D_3$ in the cytosol. $1,25(OH)_2D_3$ is a potent agent in inducing bone resorption *in vitro* and these data suggest that glucocorticoids may exert part of their effects on calcium metabolism and bone *in vivo* by directly regulating the turnover of cytosol $1,25(OH)_2D_3$ receptors in one or more types of bone cell.

We recently developed a simple method for the detection of high-affinity glucocorticoid binding ($K_D \simeq 5\times10^{-9}$ mol l^{-1}) in fetal rat calvaria cytosol[1] and, by applying a similar method we also detected binding of similar affinity ($K_D \simeq 2.3\times10^{-9}$ mol l^{-1}) for $1,25(OH)_2D_3$ (ref. 2). Glucocorticoid receptors had been previously demonstrated in bone cells by Feldman *et al.*[3], and $1,25(OH)_2D_3$ receptors in bone calvaria cytosol in a preliminary report by Kream *et al.*[4]. In the latter study bone cytosol receptors for $1,25(OH)_2D_3$ were shown by sucrose density gradient centrifugation to have sedimentation characteristics (3.5S) similar to the better-defined $1,25(OH)_2D_3$ receptors of intestine. We have confirmed this finding (data not shown). In our method, in contrast to others, the equilibrium was set up first in the intact calvaria at 4 °C and then the cytosol prepared by sonication and ultracentrifugation, and bound separated from free steroid with dextran-charcoal. The advantages of this method are its simplicity, elimination of the risk of degradation of receptors and loss of cells during collagenase digestion, and the establishment of binding to intracellular receptors at their physiological concentrations.

The bound:free ratio in the cytosol using tracer-labelled glucocorticoid or $1,25(OH)_2D_3$ (10^{-9} mol l^{-1}) is as high as 60% displacing down to 5–10% with 100-fold excess of unlabelled steroid. Cross-competition experiments with up to 1,000-fold excess competing steroid showed that the glucocorticoid and $1,25(OH)_2D_3$ receptors are quite distinct from one another.

Although only a rough assessment of K_D or binding-site concentration is possible by this method, it is highly reproducible both for glucocorticoids and $1,25(OH)_2D_3$, and we have found the binding characteristics to be similar to those obtained in cytosol prepared by the methods of Feldman *et al.*[3] for glucocorticoids and Kream *et al.*[4] for $1,25(OH)_2D_3$ respectively. The reproducibility is due in part to the very slow rate of dissociation of glucocorticoids and $1,25(OH)_2D_3$ from their respective receptors as a consequence of dilution after cell disruption, which seems not to be significant over the 1–4 h elapsing between sonication of calvaria and the final charcoal separation step. This simple method is suitable for semi-quantitative study of changes in overall receptor content during culture of intact bone.

A marked difference was observed between the stability in short-term organ culture of the rat calvaria cytosol receptors for glucocorticoids, and $1,25(OH)_2D_3$ (Fig. 1). High-affinity glucocorticoid binding persisted for 24 h unchanged, while that for $1,25(OH)_2D_3$ diminished progressively after 2 h in culture and was minimal by 24 h. This marked difference suggested to us that $1,25(OH)_2D_3$ cytosol receptor content is being actively maintained by some agent or agents *in vivo* that are missing from the culture medium.

Oestradiol or testosterone (each at 10^{-6} M) had no effect on the rate of disappearance of $1,25(OH)_2D_3$ binding. These steroids lack specific cytosol receptors[3,5] in rat calvaria. However, a marked and dose-dependent effect of the glucocorticoid cortisol was demonstrated over the range 10^{-8} to 10^{-6} M (Fig. 2). There was no consistent effect at 2 h, but a dramatic preservation of $1,25(OH)_2D_3$ binding at 6, 24 and 48 h. After culture for 48 h in the absence of cortisol there was no residual high-affinity $1,25(OH)_2D_3$ binding, while in the presence of cortisol (10^{-6} M) binding was preserved at the level seen after 24 h of culture with cortisol. When calvaria were cultured for the first 24 h without steroid, and cortisol (10^{-6} M) subsequently added and culture continued for a second 24 h, binding of $1,25(OH)_2D_3$ was not restored but showed no further fall over the second 24 h.

When other glucocorticoids were tested in this system at 10^{-7} M for 24 h, they all significantly preserved binding of $1,25(OH)_2D_3$. Their relative efficacies in preserving $1,25(OH)_2D_3$ were closely related to their relative potencies as glucocorticoids and affinities for the glucocorticoid receptors (triamcinolone acetonide > dexamethasone > cortisol > corticosterone > progesterone).

These findings strongly suggest that glucocorticoids are important for maintaining the content of cytosol receptors for $1,25(OH)_2D_3$ in bone, and favour an inhibition of degradation of existing $1,25(OH)_2D_3$ receptors, rather than the induction of synthesis of new receptors. Our present data do not define the mechanism, but suggest that it is mediated by binding of the glucocorticoid to its receptor. It is of potential physiological importance since it occurs at physiological concentrations of glucocorticoids.

The effects of glucocorticoids on bone are of clinical significance, being involved in the rapid and severe bone loss seen in Cushing's syndrome and in patients on steroid therapy. In the presence of excess glucocorticoids, intestinal calcium

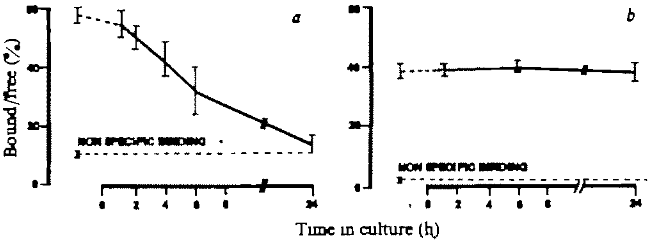

Fig. 1 Comparison of persistence of fetal rat calvaria glucocorticoid (*b*) and $1,25(OH)_2D_3$ receptors (*a*) in short-term organ culture at 37 °C. Cells were cultured on stainless steel grids, two half calvaria per grid, in sterile plastic Petri dishes with 0.3 ml culture medium under and in contact with each grid, in an atmosphere of 5% CO_2 in air at 37 °C. Culture medium was medium 199 (Flow Labs) with 1.2% $NaHCO_3$, 40 μg ml^{-1} gentamicin, 1 mM glutamine, 0.15 mg ml^{-1} ascorbic acid and 5% heat-inactivated rabbit serum (fluorometry revealed up to 1.45×10^{-8} M corticosterone in medium). All calvaria were kept at 4 °C except during the period of culture (0, 1, 2, 4, 6 or 24 h). After culture they were washed and again left in fresh culture medium at 4 °C. On completing this for the last set of calvaria, they were washed this time in incubation medium and incubated overnight in lots of four half-calvaria at 4 °C in 0.5 ml of this solution containing 1×10^{-9} M ^3H-labelled, with or without 100-fold excess unlabelled steroid. For detecting the glucocorticoid receptor $1,2,4(N)[^3H]$triamcinolone acetonide (Radiochemical Centre, Amersham, TRK 485, specific activity 29 Ci $mmol^{-1}$) was used as ligand because of its high affinity, and for the $1,25(OH)_2D_3$ receptor $23,24(N)$ $[^3H]1,25(OH)_2D_3$ (94 Ci mmol Amersham TRK 588) was used in place of the lower specific activity compound used in our earlier studies[2]. After overnight incubation, calvaria were washed again, sonicated, and the sonicate ultracentrifuged (100,000*g* for 30 min) bound and free separated with charcoal, and bound and total radioactivity counted[1,2]. Mean and range of observations of bound·free ratio from three separate experiments are indicated (six observations, each the mean of duplicates per time-point, for both steroids). The first data points are for controls at 4 °C.

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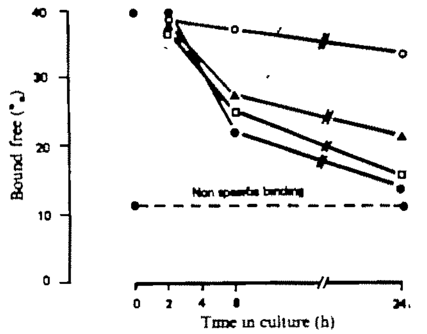

Fig. 2 Representative experiment showing the effect of including cortisol in the incubation medium on the persistence of $1,25(OH)_2D_3$ receptors in fetal rat calvaria. Each point is the mean of duplicate observations in cytosol from four half-calvaria. Incubations with 100-fold excess $1,25(OH)_2D_3$ indicate nonspecific binding which remained unchanged. In the absence of added cortisol (●) there was progressive loss of high affinity binding to very low levels at 24 h. ○, ▼ and □ indicate results in the presence of 10^{-6} M, 10^{-7} M and 10^{-8} M cortisol respectively.

absorption[6] and bone formation are reduced and bone resorption increased[7]. Numerous studies both *in vivo* and *in vitro*, in animals and man however have not fully elucidated the mechanism of these effects[8–12], although recent studies indicate that one action of glucocorticoids in birds[13] and possibly in mammals[14] is to stimulate renal 1α-hydroxylase activity. Our data indicate for the first time that glucocorticoids may influence the stability of cytosol $1,25(OH)_2D_3$ receptors in bone. From these experiments we cannot tell if this effect is specific to one population of bone cells or a general one affecting both osteoblasts and osteoclasts. There is evidence that both types of cell respond to $1,25(OH)_2D_3$ at concentrations of 10^{-9} M (ref. 15). An augmentation of $1,25(OH)_2D_3$ receptors in osteoclasts which would result from retarding their degradation might enhance bone resorption, since this metabolite of vitamin D is among the most potent bone-resorbing agents known[16,17].

Our studies indicate the need for a thorough examination of the effects of glucocorticoids on $1,25(OH)_2D_3$ and other receptors in cytosol and nuclei in isolated bone cell populations and in intestinal cells.

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Is the benzodiazepine receptor coupled to a chloride anion channel?

HIGH affinity saturable binding sites for benzodiazepines (BZs) in mammalian brain have been described[1]. These sites show stereospecificity, organ and subcellular distribution, and structure–activity relationships reminiscent of other synaptic membrane receptors which have been characterised by ligand binding[2,3]. The BZ receptor is so highly selective that no known putative neurotransmitters interact with it, thus raising the possibility that the brain contains another unidentified endogenous ligand[4,5]. Recently, Tallman *et al.* have reported that γ-aminobutyric acid (GABA) specifically enhances the binding of ^{3}H-diazepam to rat brain membranes[6]. Considerable biochemical and neurophysiological evidence suggests that GABA may be intimately involved in the mechanism of action of BZs[7–10,18]. In several neurotransmitter systems, ions seem to have specific effects on the binding of synaptic receptor ligands, presumably because of the intimate association of the receptor with an ion channel. For example, binding of opiate agonists and antagonists is differentially affected by sodium ion[11], and Na^+ conductance is altered by opiate agonists[12]. Similarly, specific effects of anions on the receptors for strychnine[13] and GABA[14] are correlated with observations that the neurophysiological action of these receptors is dependent on alterations in chloride ion conductance[15,16]. We now report the specific effect of chloride and other anions in enhancing BZ binding to its receptor. We infer that the BZ receptor (recognition site) may be

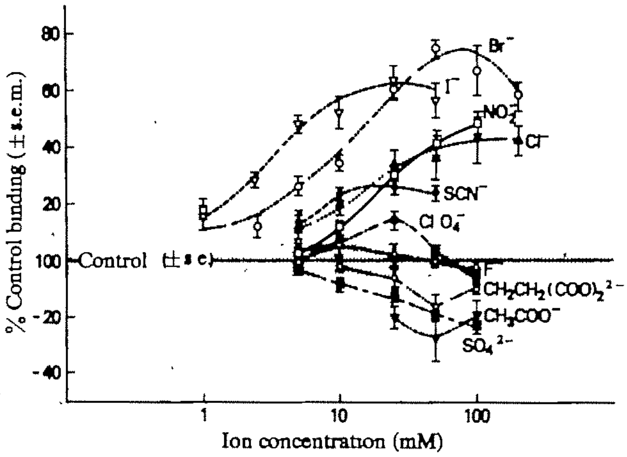

Fig. 1 Effect of various anions on ^{3}H-diazepam binding. Male rats (200–250 g) were decapitated. Cerebral cortices were washed quickly in ice-cold Tris-maleate buffer (50 mM, *p*H 7.6 at 0 °C), homogenised in 100 vol of the same buffer (w/v) using a Brinkmann Polytron for 20 s and centrifuged at 20,000 *g* for 20 min at 7 °C (J-21 Beckman centrifuge). Pellets were resuspended by Polytron in 100 vol of buffer. Radioreceptor assay incubations contained 0.5 ml of membranes, 0.3 nM ^{3}H-diazepam (~80 Ci mmol^{-1}, NEN), 0.1 ml of the added salt solution (or distilled water as control) and buffer in a total volume of 1 ml. After incubation for 30–45 min at 0 °C, tubes were rapidly filtered through glass fibre filters (GF/B, Whatman) *in vacuo*. Filters were washed twice with 5 ml of cold buffer, dried, extracted with 10 ml Aquasol (23 °C for 10 min with vigorous shaking) and counted in a Beckman scintillation counter with 40% efficiency. The *p*H of the incubation medium was unperturbed by any of the ionic additions. Anions were all added as ammonium salts, except for succinate and nitrite which were sodium salts. Nonspecific binding measured in the presence of 1 μM unlabelled diazepam was unaffected by the ions, even at the highest concentrations, and was 7.5±2% of the maximal binding. Each point indicates the mean of three determinations. Triplicate control tubes were placed at the beginning and end of each dose–response curve to detect any instability. For clarity, data for representative inactive anions have been plotted (compare with Table 1). All inactive anions produced either no significant effect on ^{3}H-diazepam binding or, more often, a small (<20%) inhibition of binding.

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Table 1 Effects of anions on ^{3}H-diazepam binding: correlation with effects on permeability

Anion	Enhancement of binding	Permeability (Eccles[15])	ED_{50} (mM)	E_{max}* (% control binding)
Iodide	+	+	2.8	160
Bromide	+	+	9.0	175–180
Chloride	+	+	13.0	142
Nitrite	+	+	20.0	148
Thiocyanate	+	+		126
Perchlorate	+/−	+		
Chlorate	−	+		
Azide	−	+		
Formate	−	+		
Fluoride	−	−		
Oxalate	−	−		
Succinate	−	−		
Acetate	−	−		
Sulphate	−	−		
Lactate	−	−		
Propionate	−	−		
Maleate	−	−		
Phosphate	−	−		

There were no significant effects of choice of anion, for example, Na^+, K^+, NH_4^+, in the case of bromide or iodide.

* E_{max}, maximal binding relative to control.

closely associated with a chloride ion channel which is also closely linked to the GABA-recognition site.

A crude membrane preparation from rat brain, obtained and incubated in an organic buffer (Tris-maleate), was assayed for ^{3}H-diazepam binding in the presence of increasing concentrations of some anions; a dose-dependent enhancement of specific binding was demonstrated (Fig. 1). Of 18 anions tested, only five proved to be clearly active—iodide, bromide, chloride, thiocyanate and nitrite. Inactive anions included organic, monovalent (formate, acetate, lactate, propionate) and divalent (oxalate, succinate, maleate), as well as inorganic, monovalent (fluoride, azide, chlorate) and divalent (sulphate). Perchlorate was active only at 25 mM. Since the dose–response curve for each of these anions differs both in regard to ED_{50} and maximal effect, it is difficult to assess a single sequence of potency. In terms of ED_{50}, iodide is the most active (2.8 mM). The maximal effect was observed with 50 mM bromide, which increases the specific binding 70% over the control. For these two anions, significant enhancement of binding was observed with concentrations as low as 1 mM. Further increase in concentration of active ions resulted in a decrease of the binding, perhaps due to nonspecific effects, consistent with the observation that most of the inactive anions tested were also slightly inhibitory at higher concentrations. The observations were independent of the cation—sodium, potassium and ammonium salts of bromide showed no statistically significant differences in their effectiveness. The effect of anions was to alter specific binding; there were no changes in nonspecific binding values for any anion tested, even at massive concentrations.

The magnitude of the anion effect is strongly dependent on temperature, being maximal at 0 °C and completely abolished at 37 °C. To characterise the anion-dependent increase of binding further, dose–response curves of the inhibition of ^{3}H-diazepam binding by increasing concentrations of unlabelled ligand in the presence or absence of iodide (25 mM) were obtained, and these data used to construct a Scatchard plot (Fig. 2). The enhanced binding was due to an increase in the affinity constant, while the number of binding sites was unchanged. Pooling data from three experiments, we obtained a mean K_d value of 3.57 ($\pm$0.3 s.e.m.)$\times 10^{-9}$ M in the absence of ion, which decreased to 2.4 ($\pm$0.13)$\times 10^{-9}$ M in the presence of 25 mM iodide. The mean binding capacity was 54 ($\pm$9.7) fmol mg^{-1} (original weight) for the control and 60 ($\pm$10.5) fmol mg^{-1} with added iodide. This latter difference was not statistically significant. The change in the apparent equilibrium constant is attributable to a change in the dissociation rate constant without alterations in the association rate constant: the $t_{1/2}$ for dissociation is 3.5 min ($\pm$0.52) for the control and 6.2 ($\pm$0.91) in the presence of 25 mM iodide (Fig. 2, inset). Association curves with or without ionic additions showed no appreciable difference in the association rate constant, but only in the plateau value due to the change in K.

The selectivity of the effective anions on binding is in excellent agreement with their selectivity for penetrating the activated inhibitory postsynaptic membrane of cat motoneurones measured electrophysiologically[17] (Table 1). Every anion which increased diazepam binding has been reported to convert the inhibitory postsynaptic potential into the depolarising direction. Of particular note is the complete inactivity of fluoride, both in the binding assay and the electrophysiological studies, in spite of the high potency of the other halides. Chlorate, azide and formate, however, failed to enhance ^{3}H-diazepam binding, but seem to pass through the chloride channel[15]. A weak anion effect might easily be masked by the nonspecific inhibitory effect (due to increased ionic strength) that we observed with all the anions tested (at high concentrations for the active ion and at lower concentrations for the inactive ions). For example, perchlorate showed an enhancement of binding at only one concentration (25 mM). Alternative explanations for the anion effect which invoke nonspecific effects (ionic strength, chaotropic effect or effects on water structure in the vicinity of the membrane) are relatively unlikely. Chlorate, for instance, is a strong chaotropic agent[19] but is inactive in this system.

The failure to find enhancement of ^{3}H-diazepam binding by anions has been reported[20], but those authors used Tris-HCl buffer, which obscures the effect.

The temperature dependence of the effect[29] which is reminiscent of the sodium effect on opiate binding[21], the change

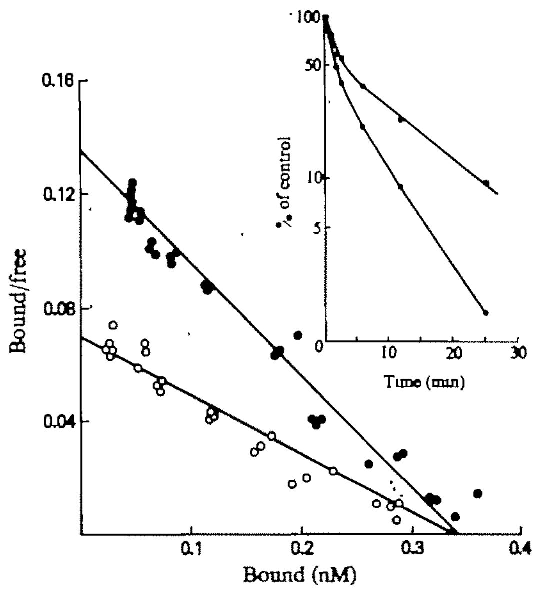

Fig. 2 Scatchard analysis of ^{3}H-diazepam binding in the presence (●) or absence (○) of 25 mM NH_4I. Assay conditions as described for Fig. 1 with a fixed amount of ^{3}H-diazepam (0.15 nM) and varying concentrations of unlabelled ligand. Data from three experiments were analysed with a weighted, nonlinear, least squares, curve-fitting program (SCATFIT)[27,28]. Binding parameters were compared within experiments using a paired analysis. In all three experiments, a major change in affinity was detected, whereas the binding capacity was not significantly affected. The equilibrium constant of association (K) was increased by a factor of 1 39 ($\pm$0.04) ($P<0.01$), and the ratio of binding capacities was 1.12 ($\pm$0.09). Inset shows the dissociation curves in the presence (○) and absence (●) of 25 mM NH_4I. The ratio of the corresponding $t_{1/2}$ values (1 77) is sufficient to explain the difference in the equilibrium constants without a change in the association rate constant.

in affinity due to a change in the dissociation rate constant, and the apparent specificity of the anions involved, all suggest that ions modulate the affinity of the BZ receptor through an allosteric-induced change of conformation. Recent experiments in our laboratory[29] have demonstrated that the GABA-induced increase in diazepam binding can be increased markedly and synergistically by including halides in the incubation medium. Since neurophysiological[22-24] and binding studies[25] already indicate an association of GABA receptor to chloride ion channels, the present data further support the concept that GABA and diazepam receptors are closely associated and may be jointly coupled to a chloride ion conductance mechanism, at least in some regions of the brain.

An interesting speculation about the nature of the endogenous ligand for the diazepam receptor may be made on the basis of analogy with the effects of ions on the opiate, GABA, and α-adrenergic receptors[26]. In these three systems, antagonist binding is unaffected or enhanced by the ion related to the conductance mechanism, whereas agonist binding is inhibited by the same ion. If this were to represent a general phenomenon, it could be predicted that the endogenous ligand for the 'Valium' receptor would be an 'agonist', while BZs themselves are antagonist compounds; in other words, the endogenous ligand should be an 'anxiety factor'.

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Transplacental effects of chemical mutagens detected by the micronucleus test

EXPOSURE to chromosome-damaging chemicals during prenatal development is particularly hazardous, since stem-cells needed to support tissue maintenance throughout life may then be at risk. Lesions in fetal cells lead to cancer in early life, and in germ-cell lineages, contribute to increased inherited abnormalities. Chemicals can be screened for chromosome-breaking potential *in vivo* by the micronucleus test[1] which measures frequencies of acentric chromosome fragments[2]. Micronuclei are usually scored in polychromatic erythrocytes from adult rodent bone-marrow. This short-term test is claimed to be as reliable as the more laborious analysis of metaphase chromosomes for chemically-induced visible aberrations[3,4]. The reactive forms of chromosome-damaging chemicals are often metabolites formed within tissues of exposed individuals. Short-term methods to determine genome-damaging potential must incorporate realistic activating systems if derived risk-estimates are to be meaningful. Although fetal organs contain enzymes which activate mutagenic/carcinogenic chemicals[5], the micronucleus test has not previously been applied to transplacental exposure. Adult bone marrow has limited activation capacity and short lived mutagens/carcinogens formed in other tissues may not survive in the circulation, so false-negative results can be obtained. Late in gestation, blood cells develop in the fetal liver, alongside hepatocytes which metabolise pre-mutagens/carcinogens to active forms. We show here that transplacental exposure to chemicals which require metabolic activation can cause chromosome breakage, indicated by micronucleus formation, in mouse fetal tissue. The dose dependence of this intrauterine genetic damage can be measured, thus providing a method for quantitative assessment of risks inherent in prenatal exposure to certain classes of carcinogenic and mutagenic chemicals.

The kinetic model presented in Fig. 1 (data from refs 6–9) predicts that transplacental and maternal effects of chromosome-damaging agents could be compared by measuring the incidence of polychromatic erythrocytes containing micronuclei in fetal liver 12–18 h after the effective exposure to the test agent, and in both maternal bone marrow and fetal blood 30 h after the effective exposure. The essential parameters on which this prediction is based are as follows. Optimal expression of chromatid and chromosomal damage occurs if the toxic agent

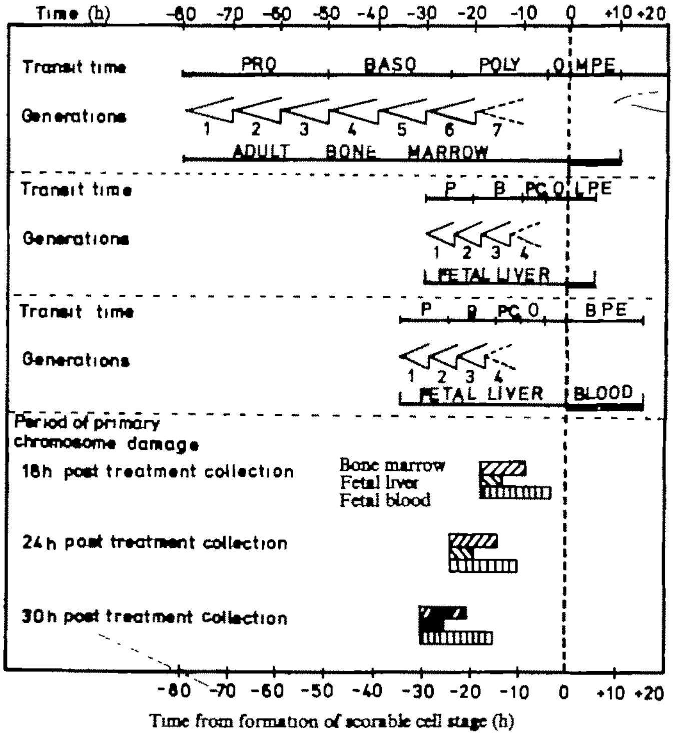

Fig. 1 Kinetic basis for optimal 'expression time' of chromosome lesions giving rise to micronuclei in polychromatic erythrocytes of maternal bone marrow, and fetal liver and peripheral blood, after treatment with chemical mutagens/carcinogens (Pro (P), proerythroblast; Baso (B), basophilic; Poly (PC), polychromatic; O, orthochromatic erythroblasts; MPE, LPE and BPE bone marrow, fetal liver and fetal blood polychromatic erythrocytes, respectively). The dotted line indicating the cell generation within the polychromatic erythroblast class is to show that some of these cells will be post-mitotic.

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reaches effective concentrations during the penultimate cell division cycle of erythroblast maturation. Adult bone-marrow erythroblasts undergo six to seven divisions, the last two occupying about 20 h. The post-mitotic cells develop for 10 h until nuclear extrusion is completed and the resulting polychromatic erythrocyte remains in the bone marrow for about 10 h. Fetal liver erythroblasts (after the 16th day of gestation) undergo four divisions, the last two occupying about 12 h. The post-mitotic cells develop for about 5 h until nuclear extrusion is completed, and the polychromatic erythrocyte remains in the fetal liver for about 5 h. Once in the fetal circulation it persists in its early form, with homogeneously distributed cytoplasmic RNA, which gives characteristic staining properties, for about 15 h.

If each target cell received damage leading to formation of a single micronucleus, the maximum incidence of polychromatic erythrocytes containing micronuclei in fetal liver and bone marrow would be ~25%. However, this maximum may not be expressed, as doses causing this degree of damage would be likely to cause other cellular lesions, and interfere with erythroid maturation.

As the daily output of early erythrocytes from the liver is about 60% of the number already in the fetal circulation, the frequency of micronucleated early polychromatic erythrocytes in the blood will be about 60% of the peak in the fetal liver.

These predictions were tested by exposing 15–16-d pregnant Swiss-albino mice to 2 mg per kg mitomycin-C. This substance, produced by the soil microorganism *Streptomyces caespitosus*, is biologically inactive in its natural state but is converted to mono- and bifunctional alkylating agents *in vivo* after reduction to hydroquinone derivatives by NADPH-dependent quinone reductase. The chromosome-damaging potential of mitomycin-C has been established in a variety of plant and animal systems and the agent has been used in the treatment of advanced cancers. The data in Fig. 2 indicate that the predictions of our model are substantially fulfilled. The dose of 2 mg per kg intraperitoneally (i.p.) (~40% of the intravenous LD_{50}) induces approximately twice the frequency of micronucleated erythrocytes in the adult bone marrow as in the fetal liver. The spontaneous incidence of micronucleated erythrocytes was 0.16±0.03% in adult bone marrow, 0.40±0.08% in the fetal liver, and 0.24±0.09% in the fetal blood.

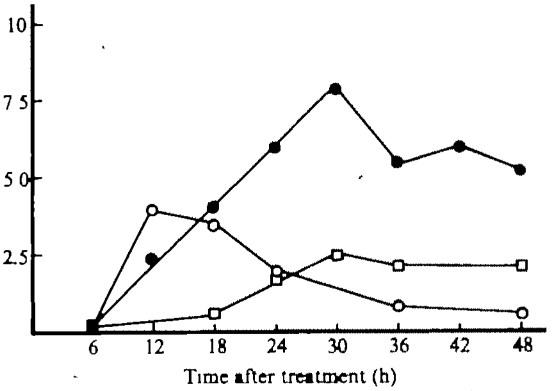

Fig. 2 Time course of incidence of polychromatic erythrocytes (PCEs) containing micronuclei in maternal bone marrow (●), fetal liver (○) and fetal blood (□) after a single i.p. administration of 2 mg per kg mitomycin-C. Data from two experiments. Total of at least four pregnant animals and two fetuses per litter examined for each point. Single cell suspensions of fetal liver and adult femoral bone marrow were prepared in freshly filtered (0.22 μm pore size) Hank's balanced salt solution +5% fetal calf serum, by repeated pipetting. Slides were prepared on a 'Cytospin' (Shandon) (4×10^4 nucleated cells in 0.2 ml per slide). Blood films were prepared directly from dissected fetuses. Slides were air dried, fixed in absolute methanol, and stained: May Grunwald (Merck) 0 25% in methanol 3 min, May Grunwald as above diluted 1:1 with Sorenson buffer, pH 6.8, 2 min; Giemsa (Merck) 14% v/v in Sorenson buffer, pH 6.7, 10 min. Stained slides were rinsed briefly in buffer followed by distilled water (total 30 s for bone marrow, 45 s for fetal liver and blood). All solutions were filtered through Whatman GFC immediately before use. This protocol maximises the distinction between polychromatic erythrocytes (blue cytoplasm) and mature erythrocytes (yellow-orange cytoplasm) and stains micronuclei red-purple. Air-dried slides were mounted in DePex. A minimum of 1,000 polychromatic erythrocytes were evaluated per slide with a 100× objective.

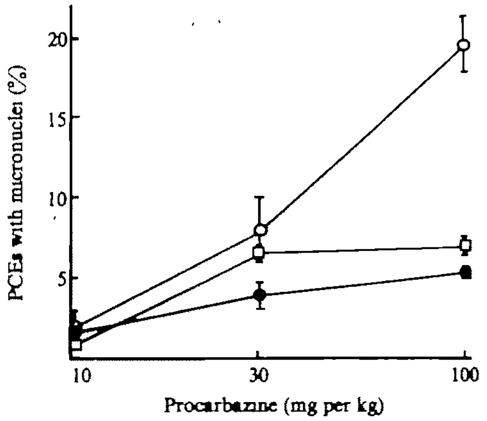

Fig. 3 Dose responses to procarbazine. Fetal liver (○), fetal blood (□) and maternal bone marrow (●) were examined for frequency of polychromatic erythrocytes (PCEs) containing micronuclei 12, 18, 24, 30 and 36 h after 10, 30 or 100 mg per kg procarbazine had been administered by single i.p. injection. At least three pregnant animals and two fetuses from each of the three litters were examined per point. Data plotted from time giving maximum incidence for each dose and tissue and expressed as mean ± s.e.

Similar tests were carried out with procarbazine hydrochloride, another anti-neoplastic drug, using single i.p. injections of 100 mg per kg (40% of LD_{50}), 30 mg per kg and 10 mg per kg. This agent is enzymatically converted to biologically active formaldehyde, methyldiazene and methyl radicals, through azo procarbazine. Activity of the three available routes (N^1 hydroxylation, N^1 demethylation or N^2 hydroxylation) may vary between tissues. Although clearly mutagenic to mammalian cells *in vivo*, mutagenicity of procarbazine cannot be reliably detected by *Salmonella* or *Saccharomyces* systems[10–12]. Figure 3 shows that dose–response curves can be constructed from all three tissues examined. Fetal liver is more damaged by procarbazine than adult bone marrow.

These preliminary experiments establish the usefulness of the erythrocyte micronucleus test applied to prenatal mice. The frequency of spontaneous micronucleus formation in the fetal liver is low, and preparations of fetal liver and fetal blood cells for microscopic observation are simply made and scored. We are extending this analysis to a wider range of environmentally significant mutagens and carcinogens, including those which have given negative results in the standard adult erythrocyte micronucleus test.

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Reduction of benzo(*a*)pyrene mutagenicity by dihydrodiol dehydrogenase

THE enigma of how inert chemicals can exert potent mutagenic, carcinogenic, allergenic and cytotoxic effects has been much debated. It has been learned that such compounds are metabolically converted to chemically reactive species[1]. In the case of aromatic or olefinic compounds, monooxygenases located in the membranes of the cell can transform these compounds into epoxides[2–5] which by virtue of electrophilic reactivity can bind chemically to cellular macromolecules such as DNA, RNA and proteins, thereby disturbing biochemical control mechanisms and leading to the above mentioned toxic effects. The same membranes in which such epoxides are produced possess an enzyme, epoxide hydratase, which is capable of inactivating epoxides[2–4]. However, with polycyclic hydrocarbons the situation is more complicated. If the epoxide groups are located on a benzo ring opposite a bay region, the resulting dihydrodiols are precursors of even more reactive dihydrodiol epoxides which readily bind to DNA[6], are often strongly mutagenic[7–10] and may be mainly responsible for carcinogenic effects of some polycyclic hydrocarbons[11]. Because, with the environmental carcinogen benzo(*a*)pyrene, epoxide hydratase was unable to inactivate these dihydrodiol epoxides[12,13], we have investigated the ability of a dihydrodiol dehydrogenase[14–16] to sequester the precursor dihydrodiols and thereby afford protection. To this end we have used bacterial mutagenesis[17] as the biological endpoint to investigate the metabolic activation of benzo(*a*)pyrene by microsomal monooxygenase. Unfortunately, the dihydrodiol dehydrogenase requires the same pyridine nucleotide cofactor as the monooxygenases, so that simple removal or addition of cofactors could not be used to study the role of this enzyme. Therefore purification of the enzyme was necessary. We show here that in metabolic situations where epoxide hydratase cannot protect against benzo(*a*)pyrene metabolite-evoked mutagenicity, pure dihydrodiol dehydrogenase reduces it. This demonstrates another factor which could contribute to differences in susceptibility to polycyclic hydrocarbon-mediated toxicity between various animal species, strains, sexes, organs and developmental stages, and to differences in potency of polycyclic compounds. This enzyme also provides an additional tool for studying the molecular mechanisms of chemical carcinogenesis.

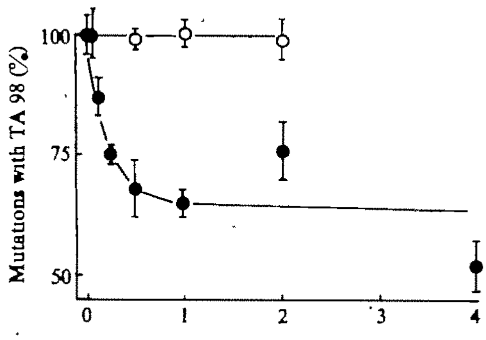

Fig. 1 Effect of dihydrodiol dehydrogenase on the mutagenicity of benzo(*a*)pyrene activated by liver microsomes from control (○) or 3-methylcholanthrene-treated (●) mice. Benzo(*a*)pyrene (5 μg, dissolved in 10 μl dimethylsulphoxide), 500 μl of a microsomal metabolising system (1 mg liver microsomal protein from adult male C3H mice, 2 mM NADP$^+$, 2 mM NADPH, 8 mM $MgCl_2$, 80 mM KCl, 50 mM Na phosphate, *p*H 7.4) 100 μl *his*$^-$ bacteria (1.7×10^8 *S. typhimurium* TA 98), various amounts of pure dihydrodiol dehydrogenase (in 100 μl 150 mM KCl) and 2,000 μl histidine-poor top agar (0.55% agar, 0.55% NaCl, 50 μM histidine, 50 μM biotin, 25 mM Na phosphate, *p*H 7.4, 45 °C) were mixed in a test tube and poured onto a minimal agar plate. After incubation for 3 d in the dark, colonies (*his*$^+$ revertants) were counted. Values are means ± s.e.m. of 3–10 parallel incubations.

Dihydrodiol dehydrogenase activity was measured by following the production of NADPH spectrophotometrically at 340 nm. The incubation, which was carried out at 37 °C, consisted of *trans*-1,2-dihydroxy-1,2-dihydrobenzene (1.8 mM), NADP$^+$ (2.2 mM), glycine buffer (50 mM, *p*H9.0) and the enzyme sample. One unit of enzyme activity was defined as the amount of enzyme which produced 1 μmol NADPH per min in these conditions. The 100,000*g* supernatant of liver homogenate of adult male Sprague–Dawley rats contained about 0.01 units dihydrodiol dehydrogenase per mg protein. The enzyme was purified about 500-fold with respect to rat liver 100,000*g* supernatant by a method which included ammonium sulphate precipitation, DEAE-cellulose chromatography, interfacial salting-in and gel filtration through Sephadex G-100. The preparation showed a single polypeptide band when analysed by SDS-gel electrophoresis and sedimented as a single symmetrical peak in the analytical ultracentrifuge in the absence of SDS. Refiltration through Sephadex G-100 resulted in a single protein peak of uniform specific activity. All these findings suggest that the preparations were homogeneous.

Metabolising system	*his*$^+$ Revertants Without DH		With DH	Decrease in mutagenicity by DH(%)
Microsomes from 3-methylcholanthrene-treated mice, NADPH generating system	2,200 ± 300	$\xrightarrow{P<0.01}$	1,300 ± 150	41
	↓ $P<0.01$		↓ NS	
As above + epoxide hydratase inhibitor	1,200 ± 300	$\xrightarrow{NS}$	1,300 ± 50	−8

Fig. 2 Effect of dihydrodiol dehydrogenase on the mutagenicity of benzo(*a*)pyrene with or without simultaneous inhibition of epoxide hydratase. Adult male C3H mice received a single intraperitoneal injection of 3-methylcholanthrene (80 mg per kg body weight, dissolved in sunflower oil) 40 h before death. The livers were homogenised in three volumes 0.15 M KCl buffered with 10 mM Na phosphate, *p*H 7.4, the homogenate was centrifuged for 10 min at 10,000*g*, and the resulting supernatant fraction centrifuged for 1 h at 100,000*g* to give a microsomal pellet. Benzo(*a*)pyrene (5 μg, dissolved in 10 μl dimethyl sulphoxide), 500 μl of a microsomal metabolising system (1 mg microsomal protein), 1 unit glucose-6-phosphate dehydrogenase, 36 mM NADP$^+$, 15 mM glucose-6-phosphate, 8 mM $MgCl_2$, 80 mM KCl, 50 mM Na phosphate, *p*H 7.4), where indicated an epoxide hydratase inhibitor[26] (0.3 μl 1,1,1-trichloropropene 2,3-oxide) and/or 0.25 units dihydrodiol dehydrogenase (DH, dissolved in 100 μl 0.15 M KCl), 100 μl *his*$^-$ *bacteria* (1.7×10^8 *S. typhimurium* TA 98) and 2,000 μl histidine-poor top agar (0.55% agar, 0.55% NaCl, 50 μM histidine, 50 μM biotin, 25 mM Na phosphate, *p*H 7.4, 45 °C) were mixed in a test tube and poured onto a minimal agar plate. After incubation for 3 d in the dark, colonies (*his*$^+$ revertants) were counted. The number of revertant colonies in the absence of benzo(*a*)pyrene (38 colonies) was subtracted. Values represent means ± s.d. of 3 parallel incubations. Statistical significance was calculated with the Student's *t*-test; NS, not significant.

The effect of this pure enzyme on the mutagenicity of benzo(*a*)pyrene was studied in the microsome/*Salmonella* test[17]. In this test two metabolic pathways mainly contribute to the mutagenicity of benzo(*a*)pyrene[12]: (1) activation to benzo(*a*)pyrene-4,5-oxide[12,18] is predominant when liver microsomes from control animals are used; (2) dihydrodiol epoxides are mainly responsible for the mutagenicity when low doses of benzo(*a*)pyrene are activated by microsomes from animals in which cytochrome P448 is induced (by treatment with polycyclic hydrocarbons or with Aroclor 1254)[12]. In this situation simple epoxides again become the major mutagenic metabolites if epoxide hydratase is inhibited, or at benzo(*a*)pyrene concentrations which are high enough to inhibit

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oxidation of the dihydrodiols. The *Salmonella typhimurium* strains TA 98 or TA 100 are highly susceptible to both groups of mutagenic metabolites, whereas the strain TA 1537 is much more susceptible to the 4,5-oxide than to the dihydrodiol epoxides[12].

Figure 1 shows the effect of dihydrodiol dehydrogenase in these two conditions. When benzo(*a*)pyrene was activated by liver microsomes from 3-methylcholanthrene-treated mice, addition of pure dihydrodiol dehydrogenase clearly reduced the mutagenicity. The effect increased with the dose of dihydrodiol dehydrogenase up to 0.5 units per plate; further addition of dihydrodiol dehydrogenase had no significant effect. Thus, maximal reduction of mutagenicity was achieved using about 15 times more enzyme than that present in the amount of liver equivalent to the microsomes used for activation. Similar, and even greater, differences in activities of foreign compound-metabolising enzymes are encountered between various animal species[19,20] or after exposure to foreign compounds with inducing properties[21,22].

The percentage of the benzo(*a*)pyrene mutagenicity which could be removed by dihydrodiol dehydrogenase depended on the bacterial strain. With TA 98, it was 30–60%, and with TA 1537, as expected from the lower sensitivity of this strain for benzo(*a*)pyrene dihydrodiol epoxides, it was smaller, 15–25% (data not shown). In contrast to these experiments with microsomes from 3-methylcholanthrene-treated mice, no effect of dihydrodiol dehydrogenase on the mutagenicity of benzo(*a*)pyrene was found when microsomes from control mice were used (Fig. 1). This is readily understandable, as control mouse liver microsomes activate benzo(*a*)pyrene mainly to benzo(*a*)pyrene-4,5-oxide rather than to dihydrodiol epoxides[12,18]. This is the metabolic situation where epoxide hydratase markedly reduced the mutagenicity[12,23]. Similarly, with 3-methylcholanthrene-induced microsomes in which the formation of dihydrodiols was prevented by inhibition of epoxide hydratase (Fig. 2), dihydrodiol dehydrogenase failed to reduce the mutagenicity of benzo(*a*)pyrene. The dihydrodiol dehydrogenase-resistant mutagenicity is probably due to simple epoxides[13,18,24,25], phenols[24,25] and metabolites of phenols[13] which are generated by the mammalian activating system.

Thus we conclude (1) that dihydrodiol dehydrogenase can protect against mutagenic effects of benzo(*a*)pyrene, but (2) that not all the mutagenic metabolites of benzo(*a*)pyrene can be removed by dihydrodiol dehydrogenase. (3) Probably due to the combination of a relatively efficient inactivation of other active metabolites of benzo(*a*)pyrene and the high inherent biological activity of dihydrodiol bay-region epoxides, the latter seem to play an especially important part in mutagenicity, DNA binding, cell transformation and tumour formation[8–11]. Therefore, the discovery that dihydrodiol dehydrogenase is capable of reducing the mutagenicity mediated by the dihydrodiol–epoxide pathway opens an intriguing perspective for the entire field of polycyclic hydrocarbon-evoked mutagenicity, carcinogenicity and cytotoxicity.

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Conformation of Moloney murine leukaemia proviral sequences in chromatin from leukaemic and nonleukaemic cells

THE structure of transcriptionally active and inert regions of eukaryotic chromatin can be differentiated on the basis of their sensitivity towards nuclease digestion. Whereas DNase I preferentially digests transcriptionally active genes into fragments too small to form stable hybrids, micrococcal nuclease shows no preferential digestion of specific nuclear DNA sequences[1–6]. We report here experiments in which the structure of Moloney murine leukaemia proviral DNA sequences in chromatin of target cells expressing the viral genome and of non-target cells in which the same sequences are repressed were examined by digestion with DNase I and micrococcal nuclease. It is shown that the Moloney murine leukaemia virus (M-MuLV) genomes in target and non-target cells of BALB/Mo mice are integrated into the host cell genome and organised in nucleosome-like structures. The difference in transcriptional activity of viral genomes in target and non-target cells is reflected by a difference in DNase I sensitivity. A partial resistance to DNase I of M-MuLV sequences in target cells suggests that the multiple viral genome copies in these cells are integrated at different sites and have different transcriptional activity.

Retroviruses provide excellent model systems for studying the structure of eukaryotic chromatin. As endogenous viruses they are normal constituents of the genomes of a wide variety of animal species. Alternatively, they can integrate into the genome of their host organisms after exogenous infection where they serve as template for the transcription of viral mRNA and progeny genome RNA (refs 7, 8). The expression of the integrated retroviral genome is subject to control by genetic factors of the host[9] and is linked to the differentiated state of the infected cell[10]. Thus, these viruses offer the unique opportunity for studying the structural organisation of well defined DNA sequences in various states of expression.

The experiments described here were carried out with nuclei isolated from different organs of BALB/Mo mice and therefore reflect the state of proviral DNA sequences in the animal. BALB/Mo mice were derived from an animal infected at the four- to eight-cell preimplantation stage with M-MuLV. They carry the M-MuLV genome integrated into the DNA of all organs and transmit it like an endogenous virus[11,12]. The genetically transmitted viral structural gene, the *Mov-1* locus, has recently been assigned to mouse chromosome 6 (ref. 13). BALB/Mo mice develop a specific, thymus-derived leukaemia. Expression of the M-MuLV genome during leukaemogenesis is restricted to lymphatic target tissues (spleen, thymus) and is

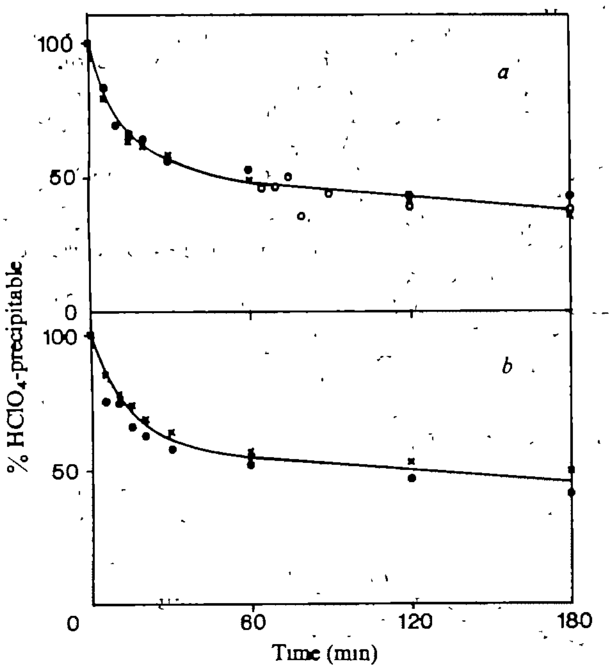

Fig. 1 Kinetics of digestion of mouse liver and spleen chromatin with *a*, DNase I, and *b*, micrococcal nuclease. Nuceli were isolated from homogenised mouse tissues by centrifugation through 2.2 M sucrose and digested at 37 °C at a nuclear DNA concentration of 1 mg ml^{-1}. Enzyme concentrations were 10 units ml^{-1}. The percentage of DNA remaining $HClO_4$-precipitable was determined by precipitation with cold 7% $HClO_4$ and subsequent measurement of the absorbance at 260 nm of the acid-soluble and insoluble fraction. ●, Spleen nuclei; ×, liver nuclei; ○, fresh enzyme was added after 1 h.

correlated with a somatic amplification of viral DNA sequences[11,14]. In contrast, the viral genome remains repressed throughout the life of the animals and no amplification of viral sequences occurs in non-target organs (such as liver or brain)[14].

We have carried out DNase I and micrococcal nuclease digestions of chromatin from target (spleen) and non-target (liver) cells of homozygous[12] leukaemic BALB/Mo mice. The conditions of digestion with both enzymes were chosen to render approximately 50–60% of the DNA $HClO_4$-soluble within 60 min. Further incubation or the addition of fresh enzyme did not significantly increase the $HClO_4$-soluble fraction, indicating that the undigested fractions were resistant to the action of the enzymes (Fig. 1). These results confirm the findings of others[1]. After different periods of DNase digestion, aliquots were withdrawn and the DNA was purified and analysed for M-MuLV-specific sequences by a quantitative complementary DNA excess hybridisation using an M-MuLV-specific cDNA which does not recognise endogenous virus sequences[12,15]. As shown in Fig. 2*a*, M-MuLV-specific sequences were preferentially digested by DNase I in spleen cells of homozygous leukaemic BALB/Mo mice: whereas undigested DNA from these cells contained three to four M-MuLV genome copies per haploid mouse genome equivalent, this number was reduced to approximately two copies after digestion with DNase I for only 10–15 min. This was not observed in liver cells from the same animals: liver cells contained one viral genome copy per haploid mouse genome, which was not preferentially digestible by DNase I (Fig. 2*a*).

The preferential digestibility by DNase I of M-MuLV-specific sequences in chromatin from spleen but not from liver cells of homozygous BALB/Mo mice agrees with the finding that the viral genome, although genetically transmitted and present in every cell of these mice, is expressed only in 'target' organs such as spleen and thymus, whereas it stays repressed in 'non-target' organs such as liver and brain[14]. However, as shown in Fig. 2*a*, in spleen cells of homozygous, leukaemic mice, in which an amplification of the viral genome from initially one to three to four copies per haploid genome had taken place, only two of the copies were sensitive to DNase I, whereas the remaining one to two copies showed the same relative resistance towards DNase I as the bulk of the host DNA sequences. In analogy to the findings of others[1–6], we assume that the DNase I-sensitive sequences are transcriptionally active, whereas the resistant ones are inactive. This assumption is supported by the preliminary finding that in young homozygous BALB/Mo mice which contain only one M-MuLV genome copy in their target cells[14], this single copy is sensitive to DNase I digestion.

The partial resistance of M-MuLV sequences to DNase I in spleen cells of homozygous, leukaemic BALB/Mo mice (Fig. 2*a*) therefore suggests a difference in the transcriptional activity of the various viral genome copies in leukaemic tissues. On the basis of these data, however, we cannot exclude a heterogeneity of the cell population in the sense that in some cells all M-MuLV sequences are DNase I-sensitive and in others all are resistant. Furthermore, as the complexity of the cDNA probe used in the hybridisation analysis has not been accurately determined[12] and nothing is known about the molecular organisation of the various M-MuLV genome copies integrated in leukaemic cells,

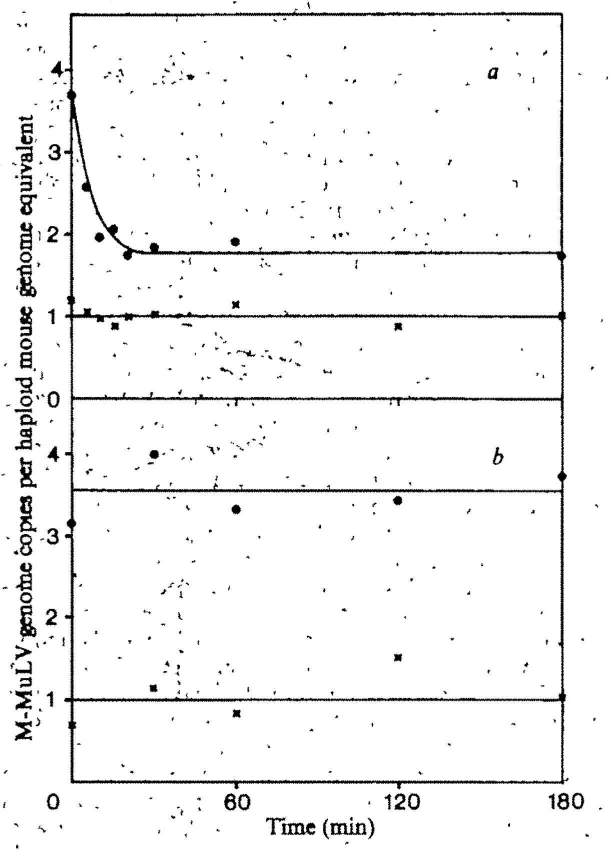

Fig. 2 Determination of M-MuLV-specific sequences in DNase-digested chromatin by molecular hybridisation. Nuclei from spleens (●) and livers (×) of homozygous leukaemic BALB/Mo mice were isolated and digested with *a*, DNase I, and *b*, micrococcal nuclease, as described in Fig. 1. After the times indicated, undigested DNA was extracted from the DNase-treated nuclei, purified by CsCl-ethidium bromide equilibrium centrifugation and boiled in alkali as described elsewhere[11,12]. The concentration of M-MuLV-specific DNA sequences was determined in each DNA preparation in a cDNA excess hybridisation assay using an M-MuLV-specific cDNA probe essentially as described elsewhere[12,15]. M-MuLV genome copy numbers were calculated by comparison with standard mouse DNAs with known contents of viral sequences (homozygous and heterozygous BALB/Mo embryo DNAs containing 1 and 0.5 M-MuLV copies per haploid mouse genome equivalent, respectively[12]). Thus, the ordinate represents M-MuLV genome copies per weight of undigested DNA expressed as haploid mouse genome equivalents.

Table 1. Hirt fractionation

DNA source	M-MuLV genome copies per haploid mouse genome	% Of total DNA in supernatant	% Of M-MuLV-specific DNA in Supernatant	% Of M-MuLV-specific DNA in Precipitate
Spleen	3.4	2.5	2.4	97.6
Liver	1.4	3.8	4.5	95.5
SV40	—	89.5	—	—

Mouse tissues were homogenised in a loose Teflon homogeniser in phosphate-buffered saline (PBS) (liver 5 ml PBS per g; spleen 20 ml PBS per g) The homogenate was diluted with 6 volumes of 10 mM Tris-HCl, pH 7.6, 10 mM EDTA, and the high molecular weight DNA was precipitated with 1% SDS and 1 M NaCl as described by Hirt[16] The precipitates were centrifuged (30 min, 27,000 g, 4 °C), DNA was extracted and purified from the supernatant and precipitate fractions, and M-MuLV-specific sequences were determined by molecular hybridisation[12,15]. In a control experiment, radioactively labelled SV40 DNA was added to a spleen homogenate to determine the degree of trapping and co-precipitation of free viral DNA with the Hirt precipitate.

we cannot determine whether parts or all of the viral genomes are in the sensitive configuration.

When similar experiments were carried out with micrococcal nuclease, we found that this enzyme did not preferentially digest viral sequences, in either target or non-target tissues, of leukaemic BALB/Mo mice (Fig. 2b): the relative concentration of viral sequences (three to four copies per haploid genome in spleen and one copy in liver cell chromatin) was constant throughout the course of digestion. This was observed reproducibly in organs from BALB/Mo mice of different age as well as in M-MuLV-producing fibroblasts *in vitro* (data not shown). This result indicates that the M-MuLV genomes in target and non-target organs of BALB/Mo mice, including the copies that are amplified during leukaemogenesis, are organised in similar nucleosomal structures to those of the host cell DNA, and suggests that the multiple viral genome copies in tumour cells are integrated into the host cell genome and not present as free proviral DNA.

To determine directly whether unintegrated proviral DNA contributed to the DNase I-sensitive fraction of the M-MuLV-specific sequences in the experiments described above, we fractionated the DNA from spleen and liver cells of leukaemic homozygous BALB/Mo mice by the Hirt procedure[16] and tested the supernatant and precipitate fractions for M-MuLV-specific sequences by molecular hybridisation. As shown in Table 1, only 3–4% of the total DNA from mouse liver or spleen cells was not precipitated by this procedure. In contrast, when radioactively labelled SV40 DNA was added before the fractionation, almost 90% of the radioactivity was found in the supernatant, eliminating the possibility that unintegrated proviral DNA was trapped and co-precipitated with the cellular DNA. When the Hirt supernatant and precipitate fractions were analysed for M-MuLV-specific sequences, we found that 96% in liver chromatin and 98% in spleen chromatin of the virus-specific DNA was precipitated with the high molecular weight cellular DNA, showing that virtually all the viral sequences are in an integrated state and that only very little, if any, free proviral DNA is present. A similar conclusion was reached by Berns *et al.* (personal communication) on the basis of restriction enzyme analysis of DNA from liver and leukaemic cells from BALB/Mo mice which indicate that the somatic amplification of M-MuLV involves new and independent integration sites.

We have shown here that M-MuLV-specific sequences in chromatin from target and non-target organs of BALB/Mo mice have a different conformation as reflected by a difference in DNase I sensitivity. This confirms that changes in transcriptional activity of eukaryotic genes correlate with conformational changes of the respective chromatin regions[1–6]. In M-MuLV and AKR virus-induced leukaemia of mice, leukaemogenesis is accompanied by a somatic amplification of virus-specific sequences[11,14,15]. The studies reported here have shown that the multiple viral genome copies in leukaemic cells are integrated into the host cell genome and that the integrated viral sequences are organised in nucleosomal structures. The partial resistance to DNase I of M-MuLV-specific sequences in leukaemic cells (Fig. 2a) agrees with the hypothesis that the viral genome copies integrated at different integration sites have different transcriptional activity. Dino and Penhoet[17] have recently come to a similar conclusion by measuring intracellular concentrations of viral RNA in murine leukaemia virus and sarcoma virus-infected cells. It will be of interest to characterise further the various integrated M-MuLV genome copies in leukaemic cells and to correlate the different integration sites to transcriptional activity and leukaemic transformation.

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Simian virus 40 specific proteins on surface of HeLa cells infected with adenovirus 2–SV40 hybrid virus Ad2$^+$ND2

CELLS infected or transformed by simian virus 40 (SV40) express SV40 tumour-specific transplantation antigen (TSTA)[1,2]. The mechanism of tumour rejection mediated by this antigen is not yet understood, but our knowledge about transplantation rejection suggests that TSTA should be located on the cell surface[3]. SV40 TSTA has a close molecular relationship to SV40 T-antigen[4,5], which is a nuclear antigen, and until now, SV40 specific proteins have not been demonstrated unequivocally in the plasma membranes or on the cell surfaces of SV40 infected or transformed cells. In contrast, in cells infected by the nondefective adenovirus 2–SV40 hybrid virus Ad2$^+$ND2, a virus known to induce SV40 TSTA[6] the SV40-specific proteins (MW 56,000 (56K) and 42,000 (42K)) are stably incorporated into the plasma membranes[7]. These proteins are coded for—at least in part—by the SV40 portion of the hybrid virus genome and correspond to the C-terminal half of SV40 T-antigen[8]. Furthermore, these proteins are immunoprecipitable from extracts of Ad2$^+$ND2-infected cells using serum from SV40 tumour-bearing hamsters[7]. Analysis of Ad2$^+$ND2-infected HeLa cells by immunofluorescence microscopy for cell surface fluorescence using hamster SV40 tumour serum, however, failed to give a positive result (W.D., unpublished). A possible

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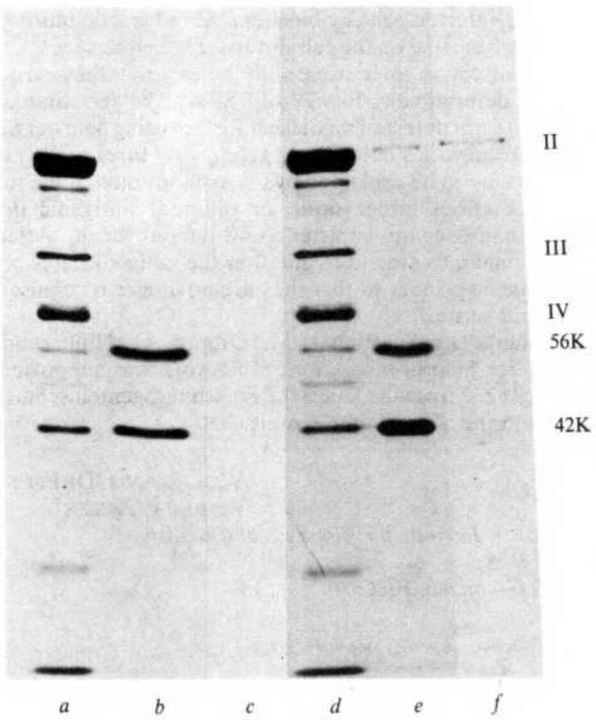

Fig. 1 Immunoprecipitation of SV40 specific proteins from NP40 cytoplasmic extracts of Ad2$^+$ND2–infected HeLa cells using hamster SV40 tumour serum or rabbit anti-T serum. HeLa cells grown in suspension were infected with Ad2$^+$ND2 and labelled from 40–41 h post-infection with ^{35}S-methionine (20 μCi ml^{-1}). NP40 cytoplasmic extract was prepared by lysing the cells with RSB[15] containing 0.5% NP40, and removal of the nuclei by centrifugation (800*g* for 5 min). Aliquots of 300 μl of the NP40 cytoplasmic extract were incubated with 30 μl of serum for 1 h at 0 °C and 150 μl pre-swelled protein A–Sepharose were added per assay and the reaction mixture was incubated for a further 30 min at 0 °C. The protein A–Sepharose containing the immune complexes was removed by centrifugation. The protein A–Sepharose was washed extensively with isotonic Tris buffer, *p*H 7.2, containing 0.2% NP40. Then the immunoglobulins were eluted from the protein A–Sepharose with 500 μl of 50 mM NH_4HCO_3, containing 1% SDS. The eluates were lyophylised and samples of the NP40 cytoplasmic extract (original reaction mixture), and the eluates were processed for SDS-PAGE analysis as described elsewhere[16]. Samples containing ~15 μg of protein were run on a 8.5% SDS-polyacrylamide gel and radioactive polypeptides were visualised by fluorography as described by Bonner and Laskey[17]. The sample order is: *a*, Ad2$^+$ND2 cytoplasmic extract; *b*, same, eluate after immunoprecipitation with hamster SV40-tumour serum; *c*, same, eluate after immunoprecipitation with hamster non-immune serum; *d*, Ad2$^+$ND2 cytoplasmic extract; *e*, same, eluate after immunoprecipitation with rabbit anti-T serum; *f*, same, eluate after immunoprecipitation with rabbit non-immune serum.

explanation is that upon location on the cell surface the structure of these proteins is altered so that they are no longer recognised by the tumour sera. These sera are probably directed against native T-antigen released from the nuclei of necrotic tumour material. We therefore decided to prepare antisera against SDS-denatured T-antigen which are directed against the primary structure of T-antigen rather than against its native conformation. We report here that SV40-specific proteins can be visualised on the cell surface of Ad2$^+$ND2–infected HeLa cells by immunofluorescence microscopy using a rabbit anti-T serum, prepared against purified SDS-denatured T-antigen.

T-antigen was prepared by preparative immunoprecipitation of cell extracts of the hamster tumour line H 65/90 B (originally obtained from Dr V. Defendi) according to the method of Schwyzer[9] using hamster SV40 tumour serum and protein A–Sepharose. T-antigen was then further purified by preparative SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretic elution of the T-antigen band. The electrophoretically pure T-antigen preparation was used for immunisation of a rabbit. The method of preparation of purified T-antigen and the immunisation procedure will be described elsewhere (W.D. and R.P., in preparation). Rabbit anti-T sera have been prepared in similar ways previously[10,11]. Hamster SV40 tumour serum (from Dr R. Henning) and the rabbit anti-T serum were first characterised by determination of their T- and U-antibody titres on SV40-transformed mouse cells (clone SV101), on heat-treated SV101 cells[12] and on Ad2$^+$ND2-infected HeLa cells[13], respectively.

Table 1 shows that both sera exhibited similar T-antibody titres on SV101 cells and similar U-antibody titres both on heat-treated SV101 cells and on Ad2$^+$ND2-infected HeLa cells.

The specificity of both sera was further assessed by immunoprecipitation of extracts from Ad2$^+$ND2-infected cells and analysis of cell extracts and immunoprecipitates by SDS-PAGE. Figure 1 demonstrates that both sera specifically precipatated the SV40-specific 56K and 42K proteins.

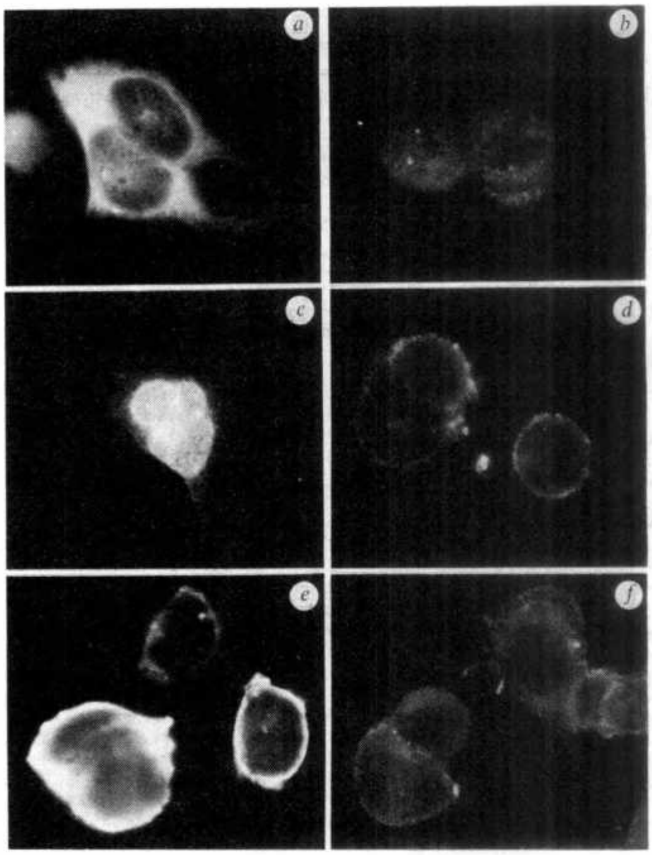

Fig. 2 Visualisation of SV40 specific proteins within and on the cell surface of Ad2$^+$ND2-infected HeLa cells by immunofluorescence microscopy. HeLa cells grown on coverslips (12 mm) were infected with Ad2$^+$ND2 (*a*, *c*, *d*, *e*) or Ad2 (*b*, *f*) and processed for immunofluorescence microscopy 40 h post-infection as described in Table 1 legend. Cells in panels *a*, *b* and *c* were fixed by methanol–acetone treatment as described in Table 1 legend, cells in panels *d*, *e* and *f* were fixed for cell surface staining by treatment for 5 min with 3.7% formalin in PBS at 0 °C followed by air drying. Cells in panels *a*, *b*, *e* and *f* were stained with rabbit anti-T serum, at a 1 : 100 dilution (panels *a*, *b*) or a 1 : 10 dilution (panels *e*, *f*) with PBS. Cells in panels *c* and *d* were stained with a rabbit antiserum prepared against purified HeLa cell nuclei. Cells were viewed with a Zeiss microscope equipped with epifluorescent illumination. Note that the uninfected cell in (*a*) (lower right) does not exhibit fluorescence staining. Cells in (*e*) are located in different focal planes. The cell in the middle right area is focused at the cell periphery, the focal plane of the island of two cells in the lower left being slightly above this. The cell in the upper middle area is either uninfected or in a very early stage of infection. Staining of either Ad2- or Ad2$^+$ND2-infected HeLa cells with rabbit non-immune serum did not result in immunofluorescence staining (data not shown). Photographs were taken with a Planapo 63 oil-immersion objective. Exposure times for panels *a*, *c* and e were ~30 s, for panels *b*, *d* and *f* ~2.5 min. Magnification in all panels is ~×366.

The rabbit anti-T serum described above was then used to analyse Ad2$^+$ND2-infected HeLa cells by immunofluorescence microscopy. Methanol–acetone fixation and staining with the rabbit anti-T serum and FITC-labelled sheep anti-rabbit-IgG revealed typical perinuclear or cytoplasmic U-antigen fluorescence[13] in the Ad2$^+$ND2-infected cells (Fig. 2*a*). This U-antigen fluorescence was found in about 80% of the cells, indicating that about this proportion of the cells were infected by Ad2$^+$ND2. Ad2-infected HeLa cells treated with rabbit anti-T serum, on the other hand, did not exhibit any fluorescence staining (Fig. 2*b*). Staining of living Ad2$^+$ND2-infected HeLa cells for cell surface fluorescence was impractical, because late in infection (~40 h) these cells are fragile and tend to lyse during processing for immunofluorescence microscopy. Analysis of Ad2$^+$ND2-infected cells for cell-surface fluorescence, therefore, was performed with formalin-fixed cells[18]. This treatment stabilises the cells and is believed not to render the cell membrane permeable to antibody. This was tested by analysing Ad2$^+$ND2-infected HeLa cells after methanol–acetone and after formalin fixation with an antiserum prepared against purified HeLa cell nuclei. Figure 2 demonstrates that this serum stained the nuclei in methanol–acetone fixed cells (Fig. 2*c*), but not in formalin-fixed cells (Fig. 2*d*). This result demonstrated that after formalin fixation antibody was no longer able to permeate the plasma membrane of the hybrid virus-infected cells. Staining of formalin-fixed Ad2$^+$ND2-infected HeLa cells with rabbit anti-T serum resulted in typical cell surface fluorescence of approximately 80% of the cells (Fig. 2*e*). This indicated that the cell surface fluorescence was associated with the Ad2$^+$ND2-infected cells. It was not visible on Ad2-infected HeLa cells (Fig. 2*f*), or when rabbit non-immune serum was used as first antibody (data not shown).

Table 1 T and U antibody titres of hamster SV40 tumour pool serum and rabbit anti T-serum

Sera	Antibody titres on SV101 cells (T-Ag)	Heated SV101 cells (U-Ag)	Ad2$^+$ND2-infected HeLa cells (U-Ag)
Hamster pool	3,200	800	600
Rabbit anti-T	1,600	1,600	600

Hamster SV40 tumour pool serum and rabbit anti-T serum were tested by immunofluorescence microscopy for T and U antibodies on SV101 cells and Ad2$^+$ND2-infected HeLa cells grown on coverslips (12 mm diameter), respectively. U antibody titres on SV101 cells were determined after heat inactivation of T-antigen[12] SV101 cells grown on coverslips (12 mm) were washed twice with phosphate-buffered saline (PBS)[14] and incubated in a wet chamber at 50 °C for 30 min. The cells were then washed twice with PBS and processed for immunofluorescence microscopy. SV101 post-infection cells, heat-treated SV101 cells and Ad2$^+$ND2-infected HeLa cells (at ~40 h.p.i.) were fixed by treatment with methanol at −20 °C for 5 min, followed by acetone treatment at −20 °C for 30 s and air drying. The cells were then incubated for 45 min at 37 °C with the first antibody (hamster SV40 tumour pool serum or rabbit anti-T serum) at the serum dilutions indicated. After washing extensively with PBS, the second antibody (FITC-labelled γ-globulin prepared against hamster IgG or FITC-labelled γ-globulin prepared against rabbit IgG, respectively) was added and the cells held for a further 45 min at 37 °C. After a second series of washes with PBS the coverslips were mounted with Elvanol on microscope slides. The cells were viewed with a Zeiss microscope equipped with epifluorescent illumination. Antibody titres are given as reciprocal of the serum dilution, at which the staining intensity was 1+ on a scale of 4+ = brilliant, and ± = very dull. Before use all sera were absorbed either on methanol-fixed 3T3 cells (when tested on SV101 cells) or on methanol-fixed, Ad2-infected HeLa cells (when tested on Ad2$^+$ND2-infected HeLa cells).

Staining of Ad2$^+$ND2-infected cells for surface fluorescence was positive with the rabbit anti-T serum down to a serum dilution of 1:100. However, when Ad2$^+$ND2-infected cells were tested for cell surface fluorescence using the rabbit anti-T serum after extensive absorption on methanol-fixed SV101 cells, a procedure which resulted in an 80% reduction of both the T and U antibody titres in this serum, no cell surface fluorescence could be obtained at a 1:10 dilution of the absorbed serum (data not shown). This result is further evidence for the SV40 specificity of the cell surface staining shown in Fig. 2*e*.

The cell surface staining of Ad2$^+$ND2-infected cells described above provides evidence that at least part of the SV40-specific 56K and 42K proteins located in the plasma membranes of Ad2$^+$ND2-infected cells by biochemical cell fractionation procedures[7] are located on the cell surface. The cell surface location of these proteins is compatible with the idea that they carry the antigenic determinants for SV40 TSTA. The fact that these proteins are not detected on the cell surface using hamster SV40 tumour serum with similar T and U antibody titres as the rabbit anti-T serum can be explained by the assumption that the rabbit serum recognises either more or different antigenic determinants than does the hamster SV40 tumour serum. Alternatively, formalin treatment might alter the antigenic sites of the SV40-specific protein so that they are no longer recognised by the tumour serum.

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Eukaryotic DNA fragments which act as promoters for a plasmid gene

TRANSCRIPTION is the process that occurs in all organisms whereby the genetic information coded by the nucleotide sequences of DNA is transferred into RNA. This process is initiated by attachment of RNA polymerase to a region of the DNA called the promoter[1]. Considerable information has accumulated recently concerning the structure and function of prokaryotic promoters, but little insight has been gained into the characteristics of eukaryotic promoters. We report here on experiments that use an *E. coli* plasmid with an attenuated promoter as a probe for possible eukaryotic promoters.

The *E. coli* plasmid pBRH3B is a 5.2 megadalton derivative of pBR316 (ref. 2) which codes for ampicillin (Ap) and tetracycline (Tc) resistance. It was constructed by inserting a chemically synthesised *Eco*RI linker into the RNA polymerase binding site (that is, the promoter) of the Tc-resistance gene, thereby decreasing the Tc resistance conferred by the plasmid from 75 μg ml^{-1} to 5 μg ml^{-1} Tc (ref. 3). Insertion of a fragment of DNA at the *Eco*RI site completely abolishes the residual activity of the resident Tc promoter, and expression of Tc resistance then becomes dependent on 'foreign' promoters contained within the inserted fragments. The level of Tc resistance thus provides a simple biological assay for the presence and possibly the strength of promoter activity carried on cloned fragments.

Purified DNAs from several prokaryotic and eukaryotic organisms (Table 1) were digested with the restriction endonuclease *Eco*RI and cloned into the *Eco*RI restriction site

Table 1 Frequency of promoter active recombinants, expressed as percentage of total recombinants

DNA source	%
E. coli bacteriophage T4	25.3, 26.2
Escherichia coli	29.3
Bacillus subtilis	25.0
Rhizobium japonicum	9.8
Euglena chloroplast	33.0
Saccharomyces cerevisiae	27.4, 28.4
Tetrahymena thermophilia	7.57
Neurospora crassa	4.74, 4.65
Drosophila melanogaster	0.77, 0.84
Megasilia scalaris	0.23

Purified DNAs from each of the organisms listed above were digested with *Eco*RI, combined with an equimolar amount of *Eco*RI-digested pBRH3B, and incubated with T4 DNA ligase[2]. The ligation mixture was transformed into the *E. coli* strain RR1 and the cells were grown for three generations (3 h) at 37°C in Luria broth (LB) after which 0.1 ml aliquots were spread on LB plates containing 20 μg per ml Ap. Frequencies of total recombinants and of promoter-active recombinants were obtained for each species of DNA by patching 500–2,000 Ap-resistant colonies onto plates containing 5, 10, 15, and 20 μg per ml Tc. Those which did not grow on 5 μg per ml Tc were scored as Tc sensitive, those growing on 5 μg ml^{-1} but no higher, as non-recombinant plasmid vehicles, and those forming colonies on higher than 5 μg per ml Tc were categorised as recombinants exhibiting promoter activity. In cases where more than one frequency is indicated, the experiment was repeated and both results are listed. In every instance the remainder of the transformation mixture was plated onto plates containing 10 μg per ml Tc and 20 μg per ml Ap in order to select only those recombinants demonstrating Tc resistance greater than the plasmid vehicle. By spreading an aliquot of the cells on Ap plates alone at the same time, we could determine the proportion of transformants represented by these promoter-active recombinants. Based on the data obtained from patching the Ap-resistant transformants, we calculated the percentage of total recombinants which showed promoter activity. The results obtained were comparable with those listed in table 1. Since each experiment was carried out in identical conditions with respect to amounts of DNA used and time of incubation after transformation, it was not necessary to adjust the data for such factors as selective advantage, sibling production and genome size.

of pBRH3B. Ampicillin resistant transformants of *E. coli* were selected and then examined for increased levels of Tc resistance. We collected a series of recombinant plasmids for each type of DNA cloned which expressed Tc resistance levels ranging from complete Tc sensitivity, to resistance greater than 5 μg ml^{-1} Tc.

For each experiment, the percentage of recombinants which exhibited Tc resistance was calculated (Table 1), and DNA from 5% of the recombinants was purified so that the presence of a foreign DNA fragment in pBRH3B could be verified by *Eco*RI digestion and agarose gel electrophoresis. A wide range of fragment sizes was observed, but no correlation between the Tc resistance levels and the sizes of the DNA fragments were found. It is evident from the data in Table 1 that the frequency of cloned promoter active fragments in pBRH3B is reproducible and depends on the origin of the DNA. Moreover, the data suggest that both yeast and *Euglena* chloroplast DNAs may harbour nucleotide sequences similar to the promoter regions present in prokaryotic DNAs. The low frequency of promoter fragments cloned from *Neurospora* and *Drosophila* DNAs stands in marked contrast to the yeast and chloroplast frequencies, and suggests that the nucleotide sequences of *Neurospora* and *Drosophila* may have evolved further from the prokaryotes than those of *Saccharomyces* or *Euglena* chloroplast DNA. Since most promoter regions sequenced thus far are usually (A+T)-rich[1], the low frequency of promoter fragments obtained from *Rhizobium* DNA in comparison with those of the other prokaryotic DNAs may be explained by the unusually high (G+C) content (64%) of *Rhizobium japonicum* 110 (ref. 4).

It is not yet certain that the regions on the eukaryotic fragments which are recognized by *E. coli* RNA polymerase are *bona fide* eukaryotic promoters. Nevertheless, preliminary indications that *E. coli* RNA polymerase can recognise eukaryotic promoter sequences come from studies involving the cloning of the *Eco*RI fragment carrying the yeast 5S rRNA gene[5] into the *Eco*RI site in pBRH3B. This fragment exhibits strong promoter activity, as manifested by the high level of Tc resistance conferred by the recombinant. When the orientation of the fragment in the plasmid is reversed, resistance to Tc disappears (unpublished observation). This observation could be explained if one presumes that one end of the *Eco*RI 5S fragment carries a promoter which can effect transcription of the Tc resistance gene. The notion that eukaryotic DNA sequences can serve as promoters in *E. coli* is further supported by studies in which functional expression of eukaryotic genes in *E. coli* was obtained. The *Neurospora crassa* gene, 5-dehydroquinate hydrolase (qa-2$^+$), when cloned in the plasmid pBR322, can complement the *E. coli* mutation, *aro*D6 (ref. 6). Moreover, similar results have been obtained with the yeast gene *his*3, which has also been cloned by complementation in *E. coli*[7]. The fact that expression of the *Neurospora* (S. R. Kushner, personal communication) and yeast[7] genes in *E. coli* is independent of the orientation of the restriction fragment, suggests that transcription is being initiated by *E. coli* RNA polymerase at some point on the eukaryotic DNA and not by a plasmid promoter.

Previous efforts to identify eukaryotic genetic control signals by probing native or reconstituted chromatin with *E. coli* RNA polymerase have yielded mostly equivocal results. The experiments described here, however, offer an *in vivo* alternative to traditional *in vitro* modes of examining eukaryotic DNA. The fact that this approach does not require the functional expression of cloned genes makes it more desirable than cloning eukaryotic genes by the complementation of *E. coli* mutations. We feel that this kind of analysis will be useful for the isolation and characterization of promoters from organisms other than *E. coli*. The strength and number of promoter active fragments cloned can be affected by many factors, so it is possible that: (1) Tc resistance levels do not accurately reflect promoter efficiency and (2) due to sibling production, the percentage of promoter active fragments shown in Table 1 may only approximate the real percentage of *Eco*RI fragments carrying promoter active sequences. It is the relative frequencies of promoter active fragments which make this system amenable to the study of the evolution of regulatory sequences from prokaryotes to eukaryotes. Indeed, the frequencies of promoter active fragments obtained from *Saccharomyces* and *E. coli* DNAs suggest a degree of relatedness greater than might be expected for two protists separated by the prokaryote–eukaryote phylogenetic barrier. To try to gain further support for this analytical approach, we are extending this survey to include DNAs from additional prokaryotic and eukaryotic organisms.

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matters arising

Viking Mars labelled release results

THE labelled release (LR) Mars experiment[1,2] yielded a positive response from Mars soil when a radioactive organic solution was added and a negative response when the soil was heated to sterilisation temperature. After storage of the soil at 10 °C for two to three months in the spacecraft, there was almost no response on addition of radioactive nutrient[3].

Nussinov *et al.*[4] proposed that LR Mars life-detection response arose from water-induced outgassing of CO_2 from Mars surface fines. They state that the kinetics of outgassing in LR and GEX are similar and that the characteristic time of the yield of O_2 in GEX and CO_2 in LR are also similar.

We are surprised that they ignored the fact that the CO_2 released by the LR is radioactive and, therefore, must have arisen from the radioactive nutrient added to the soil sample. Further, we do not agree that the reaction kinetics in GEX and LR are similar. GEX outgassed all of the measured O_2 in ~2 h whereas the half-time for the LR production of radioactive CO_2 was ~8 h, after which production tapered off, but continued slowly, for the duration of the particular experiment (up to 90 Sol).

Although we are not yet certain whether the LR response was biological or chemical, we are sure that it cannot be explained by the outgassing of CO_2 trapped in Mars surface fines.

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NUSSINOV *ET AL* REPLY—We should like to correct here a false impression that our paper[1] contained a conclusion about registration in the LR experiment of 'CO_2 trapped in Mars surface fines'. We assumed that due to the radioactivity of the $^{14}CO_2$, such a conclusion could never be drawn either by us or by readers. No conclusion as such can be made about the origination mechanism of the registered gases on the basis of the GEX and LR kinetics. It is only natural to think that $^{14}CO_2$ resulted from interaction between nutrient and O_2, the latter developing from the soil's reaction with water. The time trend of the count curve is typically filtrational which means that the formation of O_2 was rapid as compared with its transport. From a classical physical viewpoint, their kinetics implies quantitative similarity only of GEX and LR curve shapes, itself implying identity of the power dependence ($\sim t^{1/4}$ at small t) and exponential saturation ($t \rightarrow \infty$). Qualitative differences are easily explained by the fact that the very designs of GEX and LR were incorrect from the physical standpoint, namely: (1) shapes (and masses) of the GEX and LR samples were not identical; (2) specific quantity of nutrient differed in the experiments; (3) the most informative initial segments of the kinetic curves were not registered. These are the reasons that it was impossible to expect a better than order of magnitude agreement. Therefore the similarity of the GEX and LR kinetics should undoubtedly be considered as fact.

Note that the data by Levin and Straat on 'almost no response upon addition of radioactive nutrient' after storage of the soil at 10 °C for two to three months, are readily explained by our model. Indeed, O_2 physically adsorbed within the micropores at elevated temperatures can be converted to a chemisorbed state, thereby losing its reactability. At low Martian temperatures the chemisorption of O_2 is inhibited[1].

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Mechanisms of sputtering

THE letter by Pollitt *et al.*[1] on sputtering makes an interesting suggestion but also several incorrect assertions about the adequacy of existing theories and ignores other publications.

In currently accepted theories of the sputtering of metals the incident ion generates collision cascades amongst the target atoms[2,3]. If these intersect the surface, atoms receiving more than a binding energy E_b, related to the heat of sublimation, may be ejected and appear with a characteristic energy distribution having a peak at $E_b/2$ and falling off as E^{-2} at high energies of ejection E. Integration of the distribution leads to the total sputtering yield which is inversely proportional to E_b and directly proportional to the energy deposited by the incident ion in generating atomic recoils near the surface. In a crystal the lattice imposes a structure on the spread of momentum in the cascade, focuses momentum into close packed directions and leads to anisotropic emission.

The cascade lasts for a very short time, of the order 10^{-13} s. Afterwards it leaves a local heating effect in a region known as the thermal spike which may persist for up to 10^{-11} s. In cases where the density of energy is high enough and the binding energy is low enough one expects sputtering by evaporation into a characteristic energy distribution, something like a maxwellian with a peak at a few tenths of an eV[4,5]. The spike temperature and hence the sputtering yield depends on the ambient temperature of the solid[6].

These two basic mechanisms are complementary to one another, not alternatives as Pollitt *et al.* seem to suggest. The cascade component usually dominates unless the solid is at a temperature approaching its melting point, the heat of sublimation is low or the bombarding ion is of such a mass and energy as to produce cascades of high energy density. The main observations are in accord with these predictions contrary to the assertions of Pollitt *et al.*[1]. The energy spectrum of sputtered atoms closely follows E^{-2} at high energies over many decades, generally has a peak at half the sublimation energy[2,7–11] and develops a low-energy peak near 0.1 eV when thermal spike conditions are fulfilled[2,12,13]. The total yield for various elemental targets with a fixed ion and energy is inversely proportional to sublimation energy. (The dependence on ion species for fixed target is a much more complex problem involving ion implantation phenomena)[14]. From single crystal targets anisotropic emission is observed[15–17] with angular widths[18–20] and energy distributions[2,21] consistent with momentum focusing effects. The measurements of yield over several decades of ion energy, when thermal spike conditions are not fulfilled, accurately follow cascade theory[3]. They definitely do not correlate with the energy deposited in electron excitation, which they should if the model proposed by Pollitt *et al.*[1] were operating.

In insulators, and particularly alkali

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halide crystals, the characteristics of cascade and thermal spike mechanisms have also been seen[13]. But there is an additional mechanism at work which is related to electron excitation, because sputtering can be produced by low energy electrons and photons[22]. This has satisfactorily been explained[23,24] in terms of an exciton model in which a Cl_2^- ion is produced with anti-bonding orbitals which cause the sputtering, in common with the model of Pollitt *et al.*[1]. Unlike their idea it has been worked out in some detail and I am surprised they do not refer to it.

In the sputtering of adsorpates, molecular solids and polymers, experimental data are scattered. The fact that large molecular fragments are sputtered and the expectation that electronic relaxation times are relatively longer than in metals gives the model by Pollitt *et al.*[1] a better chance. An obvious test would be to search for correlation between sputtering yield and the electronic stopping power for the incident ion, perhaps by varying the ion energy over several decades straddling the energy where electronic stopping power is a maximum (say 10 keV–1 MeV H^+).

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Tectonic emplacement of ophiolitic rocks in the Precambrian Mona Complex of Anglesey

THORPE[1] believes that the Anglesey serpentinite–gabbro suite should be regarded as part of an ophiolite—and therefore tectonically emplaced—because of alleged similarities with Tethyan ophiolites. The latter embrace a large and diverse collection of phenomena, so that it is hardly surprising that some parallels can be drawn. However, other features seem to have been misrepresented by Thorpe and critical differences neglected.

Apart from occurring on a scale quite minute in comparison with Tethyan and other ophiolites, the Anglesey suite (exposed not at Newborough, but near Rhoscolyn, grid ref. 277770) does not display the diagnostic properties of an ophiolite. For example, the serpentinite and gabbro show no semblance of a layered or sequential arrangement, there is no sheeted dyke complex, and although cherty sediments and pillowed basalts do appear on Anglesey they are distant, both geographically and stratigraphically, from the intrusive suite.

Thorpe asserts that aspects of the Anglesey suite such as 'finely preserved igneous textures' near margins, and 'absence of evidence for regional thrusting' are of 'widespread occurrence' in many tectonically-emplaced ophiolite complexes'. It seems to me that many ophiolites (see ref. 2) are characterised by a peripheral tectonite fabric, at least at the basal margin, and that geological maps of ophiolite terrains are replete with thrusts.

I disagree that the suite 'provides evidence of local rapid uplift, erosion and transport'. The country rocks are, on all sides, the same, distinctive, deep-water lithology which constitutes much of West Anglesey. Debris from the suite is not found (unlike Tethyan instances). The melange (olistostrome) occurring elsewhere on Anglesey evinces such processes arising later, but it also seems to contain no fragments of the serpentinite–gabbro suite, which may have still been at depth.

I have agreed[3] that the overall Monian association suggests an ultimate oceanic or upper mantle origin for the suite, but unfortunately the geochemistry cited by Thorpe reveals nothing about how the material was emplaced. A comparison with other, such as Tethyan, ophiolites would imply emplacement by obduction, although Thorpe seems to envisage some (unspecified) mechanism associated with plate subduction. The field evidence is most compatible with the suite being a deformed magmatic peridotite–gabbro complex.

Applying the fashionable soubriquet 'ophiolite' to the Anglesey suite requires a worrying stretching of the definition, and introduces serious implications for the stratigraphy and tectonics of the Mona Complex as a whole.

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1. Thorpe, R S *Nature* **276**, 57 (1978)
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Extending the range of radiocarbon dating

THE letter from Hedges and Moore[1] on laser enrichment of ^{14}C and its application to radiocarbon dating describes a most interesting and valuable line of research deserving the acclaim of all concerned with late Quaternary studies. It may be worth pointing out, however, that their statement of their expectation that the laser enrichment and new mass spectrometric methods will allow radiocarbon dating to be applied "well into the last interglacial period"[1] begs a chronometric question for the dating of the last interglacial is not yet known with certainty. A more cautious and less pre-emptive statement might be to the effect that the new methods, by extending the far limit of radiocarbon dating to 100,000 years, may allow at least a part of the last interglacial to be absolutely dated. This is a different matter and in view of the importance of Hedges' and Moore's work the distinction is worth making clear at the outset.

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1. Hedges, R E M & Moore, C B *Nature* **276**, 255 (1978)

HEDGES AND MOORE REPLY—The distinction made by Burleigh is well taken. In this context it should be pointed out that the absolute dating to which he refers can only be achieved after reliable calibration of the radiocarbon time scale for this period. Fortunately there are grounds for believing that corrections to the radiocarbon time scale will not be very large[1].

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1. Stuiver, M *Nature* **273**, 271 (1978)

Failure to produce hepatic hyperplastic nodules in rats by portacaval anastomosis and testosterone

WEINBREN AND WASHINGTON[1] recently reported the spontaneous development of hyperplastic nodules in the livers of rats previously subjected to portacaval anastomosis (PCA). The nodules were found in all rats by 6 months post-operation. Others have failed to note such nodules in PCA rats and the authors suggested that this may be related to the difficulty in detecting the lesions microscopically or to differences in rat strains, housing or diet. We have examined 24 PCA rats 10–15½ months post-operation and have not found liver nodules. Because

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Table 1 Effects of portacaval anastomosis and testosterone in rats: autopsy data

	n	Body weight (g)		Mean change in body weight (%)	Relative organ weight (% of body weight)			Pale cells	Zone III atrophy	Bladder calculi
		Initial	Autopsy		Liver	Kidney	Spleen			
Control	9	637	659	+3.6	3.46	0.68	0.25	0	0	0
PCA (P)	9	492	475*	−2.0	2.26*	0.78	0.31‡	4	3	3
Testosterone (T)	7	661	585*	−8.4	3.77	0.86†	0.22	0	0	0
PCA + testosterone (PT)	8	486	429*	−11.6*	2.65‡	1.3	0.36‡	4	4	5
			†(PT–T)	†(PT–P)	(PT–P)	†(PT–P)				

*$P<0.001$; †$P<0.01$; ‡$P<0.05$.

Weight changes are those from beginning of testosterone treatment. P values are for comparison of treatment against control unless specified (PT–T, PT–P), using two-tailed t-test.

it is known that some androgens produce adenomas in humans[2] and facilitate the development of hyperplastic nodules in carcinogen-fed rats[3], we also tested the effect of testosterone (T) on PCA rats.

Male Wistar rats (Charles River) approximately 250 g were subjected to PCA or sham operation over a 2½-month period. Others were kept as controls. All were maintained on Purina rat chow Formulab and tap water *ad libitum* in wire bottom cages, four or five per cage. Five PCA rats killed 10 months after operation did not have nodules. Half of the remaining rats were given 200 mg testosterone enanthate (Delatestryl; Squibb) in sesame oil subcutaneously at monthly intervals for three doses. The other half received oil only (Table 1). There were six intercurrent deaths (two PCA, two PCA+T, two T); three of these animals were autopsied and found to be free of liver nodules. The remaining animals were killed one month after the last dose and 13½–15½ months (mean 14½) after operation. Under ether anaesthesia, Evan's blue in saline was injected into the spleen and the anastomosis was considered satisfactory if the liver did not darken within 10 s. Otherwise the animals were rejected from the study. Sham and control animals showed no significant difference and the data were pooled.

The livers were examined carefully for gross or microscopic nodules. None were found in any group. PCA decreased the body and liver weights and increased the spleen weights whether expressed as absolute or relative weight (% of body weight). Atrophy of zone III hepatocytes with an increased nucleo-cytoplasmic ratio in these cells compared to zone I cells was seen in 7 of 17 PCA±T rats. Scattered hepatocytes with larger vesicular nuclei and pale cytoplasm lacking the usual coarsely granular basophilia were seen in 8 of 17 PCA±T rats (Table 1). These cells were located in plates one cell thick and were not clustered to form nodules. The spleen in the PCA±T groups had follicles similar in size and character to those of controls but there were more lymphocytes and occasional plasma cells in the cords of the PCA spleens. Bladder calculi, often accompanied by pyelonephritis, were common after PCA.

Testosterone caused yellowing of the fur, gross enlargement of the seminal vesicles and a decrease in the body weights. There was a relative increase in liver weight in the PCA group and absolute as well as relative increase in kidney weight in both PCA and control groups.

PCA in this study induced liver atrophy but not hyperplastic nodules. Strain and dietary differences are likely explanations for the discrepancies between this work and that of Weinbren and Washington.

We thank Dr Fred Plum for the animals.

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On codon usage

THE total nucleotide sequence of the *lac* repressor gene *I* (361 codons assigned) has recently been determined by Farabaugh[1]. He has found that the codon usage is quite different from that of bacteriophage MS2 and ΦX174 (1,068 and 1,346 codons assigned, respectively). Nevertheless, assuming that both the repressor protein and the phage proteins are synthesised rapidly, he concluded that the codon usage presumably does not reflect a difference in efficiency of translation. As mentioned also by Farabaugh, however, no firm data actually exist on the relative translational efficiency. Moreover, even a small effect, difficult to reveal by direct methods, can have a strong selective value when judged over many generations.

Bacteriophage MS2 produces a burst size of 5,000 to 10,000 plaque-forming units (PFU) per cell, and each virus particle contains 180 coat protein molecules. Consequently, we have argued before that MS2 has optimised translational efficiency, especially of the coat cistron: one of these ways, among others, is by the proper choice of degenerate code words[2,3]. It is likely that there exists an optimal interaction energy between the codon and the cognate anticodon. As a result, codons of the type (A/U)–(A/U)-Y—where the first and second letters give an intrinsically weaker interaction—would be stabilised if the third letter is C. Indeed, in MS_2RNA the N-N-C codon is preferred over N-N-U for all codons of this type (Phe, Ile, Tyr and Asn)[4]. Conversely, intrinsically strongly interacting codons of the type (G/C)–(G/C)-Y should be loosened by a preference for U. In fact, N-N-U codons are preferred over N-N-C for Pro, Ala and Gly (Arg codons are not included as they are recognised by an inosine-containing tRNA).

When we now consider the codon usage in the *lacI* gene, which makes the *lac* repressor protein at very low levels, we find exactly the opposite situation. U as a third letter is preferrable to C for codons of the weak interaction type (Phe, Ile, Tyr and Asn); this is remarkable, considering that of third letters 28.6% are C and only 21.1% are U. Conversely, for the codons with a strong interaction in positions 1 and 2, the third letter is preferably a C rather than a U (Pro, Ala, Gly). This selective pattern of codon usage in the two systems is very striking and it would be of considerable interest if it could be related to a difference in translational efficiency. Calos[5] has shown that the low level of *lacI* expression can be explained by a low-efficiency promoter; as pointed out by Müller-Hill *et al.*[6], one can also easily imagine that the low level of constitutive *I* expression is further reduced by some limiting step in the translation processes, such as selective codon usage.

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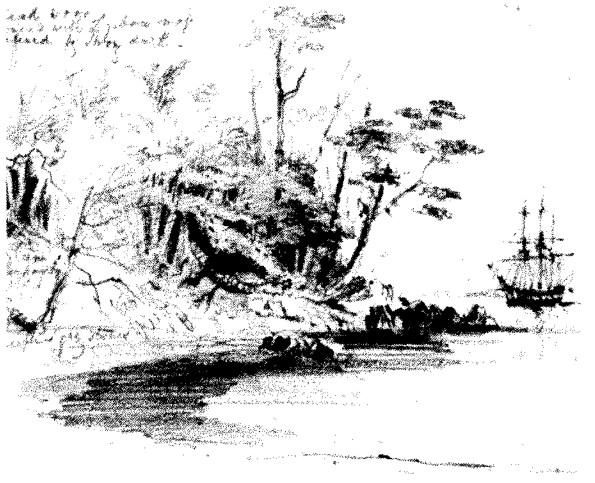

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Nature/nurture between the Wars

William J. Schull

The Triumph of Evolution: American Scientists and the Heredity/Environment Controversy, 1900–1941. By Hamilton Cravens. (University of Pennsylvania Press: Philadelphia, 1978.) $17.50.

HAMILTON CRAVENS' *The Triumph of Evolution* describes the sequence of events in the United States between the two World Wars which he contends led to the resolution of the heredity–environment controversy. Whether, indeed, a resolution has occurred, as he asserts, may seem dubious to one who follows *Nature's* book pages and the controversy over the roles of nature and nurture in differences in intelligence, or whatever it is that intelligence tests measure. Resolution here, however, merely means the achievement of an agreement among natural and social scientists that nature and nurture are distinct but interdependent variables.

The central theme of this book is that the sudden increase in the last decade of the nineteenth century of experimental biology and psychology, aided by the emergence of the new graduate universities, fostered an academic professionalisation of these sciences which had and continues to have profound repercussions. It led to changes in educational emphases in these institutions and encouraged the development of the accoutrements of science which we now take for granted. Numerous professional societies and journals were born, and a scurry for research resources begun which persists unabated. Out of this process, Cravens maintains, came consensus. A little lamented casualty was social Darwinism, at least in its most strident forms, whose adherents waned as the new professionals grew in number.

A complicated, somewhat conjectural but nonetheless intriguing series of events and developments in anthropology, genetics, psychology and sociology is set forth to support this central thesis. Much of Cravens' argument emerges through an analysis of the actions and inactions of the prominent figures of the time in the aforementioned disciplines. Some of these individuals intrigue him more than others. Franz Boas is such a case, but Davenport, Morgan, Ross, Yerkes, Dewey, Watson, Thorndike and Cattell —to mention but a few others—have their moments of attention. The activities of these individuals and their students are often interpreted in terms of their ethnic, religious and social affiliations. Their contributions to their branches of science as well as their academic and social fates are briefly limned. Most are treated sympathetically with possibly Charles Davenport emerging as the most prejudiced, least principled and least qualified of the major personalities. Familiar lesser figures such as Madison Grant, Harry Laughlin and Henry Goddard receive short shrift as is their due. To these three are ascribed some of the more sordid chapters in the eugenics movement in the United States and rightly so.

Cravens' is not the first analysis of many of these events. Kenneth Ludmerers' *Genetics and American Society: A Historical Appraisal* (1972) and Mark Haller's *Eugenics: Hereditarian Attitudes in America Thought* (1963), for examples, trace many of the same developments, and often somewhat more thoroughly but from quite a different perspective. This book makes more of the events which were occurring in the new universities, their strivings for distinction among their peers, and the emergence of a scientific community in the New World not wholly dependent upon that in the Old for its directions and philosophy. It prompts thought on the unusual impact that a few forceful personalities may have upon a discipline; witness Boas' imprint on anthropology.

Cravens' analysis does not strike me as especially disputatious. It is thoughtful and built on a serious consideration of a variety of materials—biographies, letters, journals, society proceedings and the like. Few will be able to read *The Triumph of Evolution* and not be exposed to new facts or facets in this controversy. The geneticist will learn of the turmoil his science provoked in the social sciences, and should be able to appreciate positions which may have merely seemed obdurate in the past. Similarly the cultural anthropologist, say, should learn more of the history of twentieth-century genetics, and the origins of its collective perspective. All will be exposed to parallelisms of which most readers will have been previously unaware. Many will be tempted to read further, and the extensive notes with which the book concludes will prove invaluable.

This otherwise interesting and generally unexceptionable book is marred in my view by two shortcomings. First, it is unnecessarily repetitive. Much of the contents of the chapter "The New Biology" is repeated elsewhere. We are told, for example, on p24 of G. Stanley Hall's strengths and weaknesses as the President of Clark University and much the same material appears again on p65. Edward East receives similar double treatment on pp51 and 169. Others fare in like manner. There is moreover a tiresome repetition of labels which brings me to my second objection. *The Triumph of Evolution* is flawed by the frequent use of stereotypes of people and institutions. We read of the white, Anglo-Saxon, Protestant; the left-liberal; the gentle Quaker and the humble normal school. While these may be handy labels and often serve a useful, albeit pejorative purpose in political tracts, they seem out of place to me in an historical account of this nature. H. J. Muller, for example, is variously characterised as brilliant, a radical, a man with left-liberal tendencies. Most who knew him would accept all of these appellations as valid. But he could equally well have been described as an elitist, an uncommonly gifted investigator with an incredible biological intuition, or a teacher who could communicate the excitement he found in genetics to students and colleagues alike. But do any of these labels provide a compelling basis for characterising him as one who was "quite willing to tolerate the eugenics movement and to perhaps even support some of its goals so long as the movement appeared disinterested, scientifically credible, and dignified"? I'm not questioning the attribution but merely its justification on no more forceful evidence, seemingly than a series of labels. These are poor substitutes for a penetrating analysis of the man. ☐

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Microtubules

Microtubules. By P. Dustin. Pp. 452. (Springer: Berlin, Heidelberg and New York, 1978.) DM148; $74.

It is now fifteen years since Slaughterback first coined the term 'microtubule', and the literature has grown expodentially from a mere two papers in 1963 to several hundred per annum today. During this time, there have been intermittent review chapters and specialised monographs originating from symposia, but never a survey encompassing the whole field. Finally, Dustin has written a book which deals exclusively with microtubules, which should be in every library, and which will be obligatory reading for everyone interested in microtubules.

The first half of the book is primarily concerned with the structure and biochemistry of microtubules, the interactions between microtubules, and microtubule inhibitors, whereas the second half concentrates on aspects of cellular physiology in which microtubules have been implicated, such as movement, secretion, axonal transport, and of course, mitosis. The book is not without its faults: it is for example exclusively a summary of the extensive literature and is not a critical appraisal of the work. Furthermore, it has taken two years to produce since Dustin completed his search of the literature, and so inevitably many current ideas are absent. One example is that the book only briefly mentions microfilaments and lacks any discussion of a possible interaction between microfilaments and microtubules. As such it is interesting to see where we were at two years ago, in the same way that the author's earlier book (with Eigsti), *Colchicine*, is a fascinating account of the field a decade before the visualisation of microtubules. A more serious fault is the review of the literature concerning the biochemistry of microtubules and the kinetics of assembly/disassembly *in vitro*, which is superficial and which fails to relate the properties *in vitro* to the *in vivo* behaviour of microtubules, yet clearly our understanding of the role of microtubules in cellular events will develop from such comparative studies.

Finally, the book is written as a series of independent chapters, each with its own bibliography, with the consequence that it is difficult to search for specific references. Despite these reservations, this is a book that should be read by all cell biologists. **Roy Burns**

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Autoimmunity

Autoimmunity: Genetic, Immunological, Virologic and Clinical Aspects. Edited by N. Talal. Pp 734. (Academic: New York, San Francisco and London, 1977.) $48.

Autoimmunity was considered thirty years ago as a contradiction in terms. If immunity, then regarded almost entirely to be due to antibodies, eliminated or neutralised microbes and other foreign substances which had penetrated the body it would be terribly dangerous to make antibodies against one's own tissues. Furthermore, no-one had convincingly shown that antibodies against self components were normally ever made. Antibodies could indeed be induced which reacted with an animal's own spermatozoa, lens protein or even brain tissues, but it was argued (sometimes correctly) that these were exceptions, as the antigens involved were in fact excluded from contact with cells of the immune system; and when brought into contact by infection or by injury they were essentially foreign. With improved techniques, however, undoubted autoantibodies were described in an increasing number of human diseases, such as haemolytic anaemia, thyroiditis, pernicious anaemia and rheumatoid arthritis. Autoantibodies became respectable, although it was still uncertain in most cases whether they caused the disease or were a secondary consequence of tissue damage, which had resulted in liberation of specialised tissue components that were normally hidden intracellularly but were recognised once they entered the blood stream.

The notion that autoimmunity did not occur—expressed by Ehrlich's term 'horror autotoxicus'—had been so widely accepted that few had bothered to question how the body could distinguish self from not self. In terms of current biochemical knowledge the phenomenon was inexplicable, but the clonal selection theory put forward by Burnet in 1959 made sense in terms of cell populations. It proposed that all lymphocytes potentially able to make antibodies against self antigens were eliminated if they met them during foetal life, and that auto-antibodies were the products of 'forbidden' clones, which had undergone somatic mutation after the period of obligatory development of self tolerance was over. This explanation had the added advantage of accounting for the increasing incidence of autoimmunity with age. However, in immunology few hypotheses remain intact for long. It was found that normal animals could quite easily be induced to develop autoantibodies against thyroglobulin by immunising with cross-reacting thyroglobulin, that inbred strains developed anti-thyroid antibodies spontaneously, and that normal animals contained lymphocytes with surface immunoglobulin receptors specific for thyroglobulin (and for other self components) even though they made no detectable antibodies. This implied that a considerable number of forbidden clones could not have been eliminated.

New light was shed when it was realised that specific T lymphocytes, recognising the 'carrier' part of antigens, are required to cooperate with B lymphocytes for the latter to secrete antibodies against a particular antigenic determinant. T lymphocytes are more easily inactivated by contact with antigen; the specific helper T lymphocytes having been eliminated, the B lymphocytes able to make autoantibody would only do so if an alternative set of helper cells were available. These could be provided by stimulating with cross-reacting antigens or self antigens altered in some way, such as by viral infection. Absence of autoimmunity could now be attributed to lack of helper T cells. Next came the recognition of the existence of suppressor T

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W.S. JOHNSON, Stanford, reviewing Volume 5 (Biological Compounds; edited by E. Haslam, Sheffield):

> *"To a synthetic organic chemist the chapters are not only comprehensible but interesting and highly informative . . . This is a first-class treatment of the subject . . . . Haslam and his colleagues are to be congratulated for producing such a magnificent segment of the Barton-Ollis magnum opus which indeed represents a major contribution to scholars of organic chemistry."*

J.E. BALDWIN, FRS, Oxford, is reviewing Volume 6 (Author, Formula, Subject, Reagent, Reaction Indexes; edited by C.J. Drayton, Oxford) and his review will be published shortly.

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cells, which could prevent helper cells from being stimulated or from functioning. Certain strains of animal unusually prone to autoantibody formation were found to show premature loss of suppressor cell function, and the regulatory role has been shifted to suppressor cells. Instead of self-reactive cells being absent we now have to consider them as normally present, but kept in check by an elaborate feedback system. Jerne's network hypothesis, whereby any lymphocyte specifically responding to an antigen may stimulate a specific response against itself, and so on, provides a theoretical model whose usefulness is being increasingly recognised and validated.

Against this background an up-to-date book on autoimmunity was needed. The contributors to the present multi-author volume are all experts. The topics covered in 23 chapters range from genetic control of autoimmunity in man and other species (including an interesting account of the history of NZB and NZW mice); ways in which autoimmunity can come about covering examples illustrating all the various mechanisms discussed in the introductory part of this review; role of suppressor cells; relevance of the network theory; other feedback mechanisms, including blocking factors; correlation of autoimmunity with malignancy, immunodeficiency and with a variety of viral infections; special aspects of autoimmunity, including SLE, rheumatoid arthritis, diseases of the central nervous system, and antibodies against physiologically important receptors in thyrotoxicosis and myasthenia gravis; a very full account of the pathological effects of immune complexes; and a final chapter on how autoimmune (anti-idiotype) responses can be used for positive and useful regulation. The level is advanced, but the language is reasonably clear and the book should be useful for clinicians as well as professional immunologists. The coverage is not complete (for example, there is little about haemolytic syndromes, skin diseases or the gut) but the aspects covered are done well. Bibliographies are more than adequate, and there is a reasonably good index. This book is the best and most up-to-date currently available.

J. H. Humphrey

J. H. Humphrey is Professor of Immunology at the Royal Postgraduate Medical School, Hammersmith Hospital, London, UK.

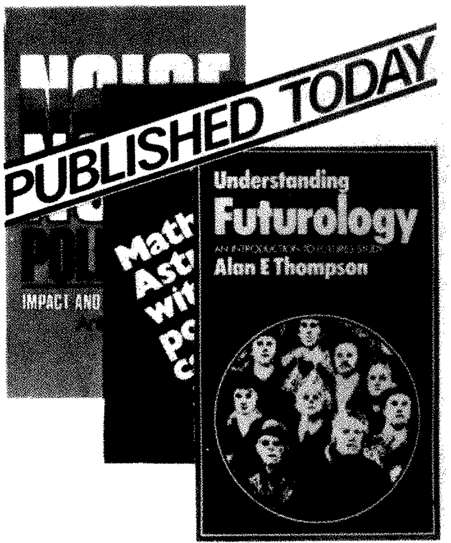
Nuclear power engineering

Nuclear Power. Vol. 1 : Nuclear Power Plant Design. Vol. 2: Nuclear Power Project Management. By Erik S. Pedersen. (Ann Arbor Science : Ann Arbor, Michigan; Wiley: Chichester, UK, 1978.) £18.60 each volume.

IN recent years nuclear engineers have undoubtedly suffered from a surfeit of excitement. After the oil shock of 1973 nuclear engineers were acclaimed as potential saviours, and almost simultaneously accused of plotting the obliteration of the planet. Nuclear engineers have seen their industry expand at breathtaking speed, only to watch now as it threatens to subside like a punctured balloon. Nuclear engineers hear politicians proclaiming the wonders and prestige of nuclear enterprise, while ducking as the groundlings throw eggs. Nuclear engineers might well welcome a little boredom. If so, may I prescribe two volumes of Erik Pedersen, taken at bedtime?

Given a topic as potentially engrossing as nuclear energy—fascinating phenomena, virtuoso engineering, daunting challenges of every kind to be met—it cannot have been easy to write 950 pages of unrelenting tedium. But both volumes of Pedersen's *Nuclear Power* read like computer printouts, an avalanche of factual information recited in a dogged monotone, in which even the most ingenious intricacy is embalmed lifeless on the page. Indeed both volumes of *Nuclear Power* read disconcertingly like each other. The first is called *Nuclear Power Plant Design*, the second *Nuclear Power Project Management;* but perhaps one-quarter of the text in the second volume is lifted verbatim from the first, without any apparent indication of this repetition. The unwary purchaser may react more emotionally to the text when he realises belatedly that he has unwittingly paid for two helpings of it.

It is not, in any case, clear just who is expected to read the books. The Preface to each volume opens with the statement that it "is intended to be used as a working reference book for management, engineers and designers, and as a graduate-level text for engineering students. The book is designed to combine theory with practical nuclear power engineering and design experience, and to give the reader an up-to-date view of the status of nuclear power and a basic understanding of how nuclear power plants function". A much better writer than Mr Pedersen would be hard pressed to find a level at which all these disparate objectives could be even minimally met. As a result both volumes of *Nuclear Power* include far too much material that



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would go far over the head of the reader desiring "a basic understanding"—unexplained references to "eigenvalues" and other jargon, and some of the most hair-raising equations *ex machina* ever put to paper, with no hint of derivation or underlying assumptions—while omitting to provide essential detail which any responsible designer, engineer or manager would require before making use of much of the information in the books.

Volume 1 opens with a chapter on nuclear reactor theory, which at once undermines the reader's confidence by getting a well-known number wrong: the Trinity test took place not on 6 July but on 16 July 1945. It may be merely a typographical error, but any book with as much undefended numerical data as this cannot afford such a demoralising error on page 2. This and subsequent chapters might serve as a recapitulation for those who already know the subject and simply need to have their memory refreshed; but the explanations will require much expansion by any student using the book as a first-time text. The questions at the chapter-ends might have been drawn from the author's draft outline: "1. Explain the buildup of atomic structure. 2. Explain the atomic number and mass number. 3. What is an isotope? . . ." and so on. A student could regurgitate the foregoing text verbatim—but might not have the faintest idea of its meaning. The chapter on 'Nuclear reactor design' uses the word 'design' as a noun, not as a verb; nowhere is there any effort to discuss the actual process of nuclear design. Instead there are reams of description, naming of the parts There are chapters on 'Types of nuclear power plants' (mostly repeated in Volume 2) and licensing (ditto, and entirely taken up with US procedures), on shielding, containment, steam and turbine cycles, electricals, instrumentation, and radioactive waste management. Volume 2 also includes chapters on safety analysis, project services, quality assurance, scheduling and plant operation, nuclear fuel management and plant cost management. Appendices list terminology and US regulatory guides. Unfortunately the index is skimpy, making the books less useful even as a concise nuclear encyclopaedia.

The economic information is alarmingly erratic. "The annual fuel expense equals about $25/kWe, which, over 30 years of plant life, implies expenditures of $750/kWe for nuclear fuel": only if you buy all the fuel at the beginning, or ignore the entire process of discounting and the economic track record of the civil nuclear industry. Similar flat statements of purported economic fact could be disputed at great length. So could the declaration of faith with which the author concludes the books: "Nuclear power is the safest, cleanest and least expensive energy source available today and in the foreseeable future. We must, therefore, take advantage of it." Given the losses so far sustained by those engaged in civil nuclear power worldwide, it is not clear who is taking advantage of whom. However, it is unhappily clear that these two volumes will do little to raise the flagging esprit de corps of Mr. Pedersen's colleagues, nor entice fresh blood into the enterprise.

Walt Patterson

Walt Patterson is energy consultant to Friends of the Earth (London) Ltd.

Annelid physiology

Physiology of Annelids. Edited by P. J. Mill. Pp. 683. (Academic: New York and London, 1978.) £28; $58.

THIS book should probably be compared with the Annelid volume of Florkin and Scheer's series on Chemical Zoology, the closest thing we have had until now to a volume on annelid physiology. Happily, only 5 of the 14 chapters in Mill's volume cover topics previously reviewed, and only two of these are by the same authors (Clark on systematics and Oglesby on osmoregulation). Comparable reviews must otherwise be sought in volumes devoted to specific physiological systems (for example, respiration and reproduction); there, in fact, they may be somewhat more useful, as physiologists are more often interested primarily in their physiological specialty and less frequently interested in results in all fields of physiology from a specific group of animals.

The volume has unfortunate technical flaws (smeared print on a number of pages in my copy) and numerous distracting errors in grammar, punctuation, and figure labelling (especially in the editor's chapter) which detract from the flow of the text. These are in addition to frequent, obvious typographical errors.

Richards' chapter on the epidermis and cuticle is a welcome summary, although more might have been included on gland cell ultrastructure, and several erroneous statements on seta formation in reproductive forms of polychaetes are included. Mill provides a list of most of the information available on sense organs and their associated pathways, but offers little synthesis.

Tashiro and Kuriyama's mistitled chapter on neurosecretion is really a good review of synapses and neurotransmitters as they occur in annelids. Annelid neurosecretion in the neuroendocrine, peptidergic sense has been reviewed several times in recent years, but neurotransmitters have been somewhat neglected; thus, the chapter is more useful than the title would lead one to believe.

Olive and Clark's chapter on reproduction nicely complements the only other recent review of this subject. Division of annelid species into monotelic and polytelic groups (that is, those which breed only once and those which breed more than once) provides an interesting touchstone especially for the analysis of polychaete life cycles. It particularly illuminates the various possibilities for physiological control. Polychaete diversity is as evident in reproduction as it is in feeding habits and external structure and this classification offers an opportunity to analyse this diversity on a rational basis.

In separate chapters, Weber discusses both respiration and the respiratory pigments. His approach is functional and he provides a good summary of the abundant recent work, especially on the pigments and their function. The diversity of polychaete life styles and respiratory pigments provides interesting material for the investigation of physiological and biochemical adaptation, and enough work has now been done in this area to sketch the broad outline of different probable physiological strategies in different respiratory stress situations. Mangum provides a critical, constructive review of the related area of temperature adaptation. These chapters emphasise polychaete diversity and will doubtless command a good audience.

In an unusual chapter on defence mechanisms, Dales reviews several neglected, and somewhat disparate topics including wound-healing (a neglected component of regeneration), responses to both parasites and microorganisms, and, briefly, graft rejection and immunity.

Oglesby's exhaustive and critical review of salt and water balance physiology again emphasises polychaetes and their diversity. This chapter ought to stand for some time as the definitive statement of our knowledge in this area.

Considering the cost of a volume such as this, both the reader and the contributing authors have a right to expect better quality from both the editor and the publisher.

Paul C. Schroeder

Paul C Schroeder is Associate Professor of Zoology at Washington State University, Pullman, Washington.

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obituary

Manne Siegbahn, 1886–1978

MANNE SIEGBAHN, who died on 24 September 1978 at the age of nearly 92, was one of the great leaders in the development of experimental atomic physics during the first half of this century. He is known in particular for his pioneering work in X-ray spectroscopy, for which he was awarded the Nobel prize in 1925, and as an eminent organiser of research in experimental physics in Sweden, where he created and led very productive research groups at Lund (1914–1923), Uppsala (1924–1937) and Stockholm (1937–1964).

Siegbahn began his academic career in 1906 at the University of Lund where J. R. Rydberg was then professor in physics. His dissertation in 1911 (on magnetic field measurements) had no connection, however, with Rydberg's famous work on series in atomic spectra, nor had it any bearing on Siegbahn's future line of research. At that time, atomic physics was in a state of rapid development. In 1913, Bohr's model of the atom, based on Rutherford's nuclear atom and the radiation laws of Planck and Einstein, had quantitatively explained the hydrogen atom, but no clear path through the jungle of amassed data on other atomic spectra was in sight.

A more promising way of getting an insight into atomic structure seemed to appear when Moseley, in 1914, by using the newly discovered Bragg reflection from crystals was able to measure the wavelengths of the X-rays emitted by different elements and found their very regular dependence on the atomic number. Siegbahn saw the possibilities of this approach and immediately put the available resources of the laboratory in Lund into the development of X-ray spectroscopy. Showing for the first time his remarkable aptitude for instrument design Siegbahn developed to perfection the crystal spectrographs and the X-ray tubes as well as the vacuum technique which was a big experimental problem in those days. He transferred his own enthusiasm to his group of students, and the laboratory in Lund became a centre for X-ray spectroscopy which attracted researchers from many countries.

As a result of a decade of hectic activity the knowledge of the X-ray spectrum of different elements was enriched with many details, the range extended toward short and long wavelengths within the limits set by the crystals, a new series (M) was discovered and the wavelength measurements were improved step by step to a high degree of precision. For practical reasons the wavelengths had to be expressed in a special (Siegbahn) unit based on an assumed value for the lattice constant of the crystals. The experimental technique was described and the observational data collected in Siegbahn's book *Spektroskopie der Röntgenstrahlen* that went through several editions, each time revised and extended, and became the bible for workers in this field.

Having been acting professor and head of the laboratory since 1915 Siegbahn was appointed to the chair in 1920 after Rydberg's death. In 1923, however, Siegbahn accepted an invitation to the chair of physics at the University of Uppsala, where he soon assembled a new group of devoted students. Although X-ray spectroscopy remained for some time the main interest, other more or less related activities were initiated. Among them was the determination of the fundamental atomic constants, leading, for example, to a considerable revision of Millikan's oil drop value for the atomic unit of electric charge.

Around this time it had been shown that X-rays could be diffracted by ruled gratings if the rays made a small enough angle with the reflecting surface (grazing incidence). Realising the possibility offered by this method to extend the observable range of X-ray spectra, Siegbahn built in 1928 a grazing-incidence concave-grating spectrograph which had the characteristic features of a Siegbahn design: it was simple, efficient and easy to work with. It proved its value and was followed by several similar instruments of increasing size. Besides amply fulfilling the hopes of extending the range of X-ray spectroscopy, these instruments effectively opened up the almost inexhaustible field of investigations into the 'optical' spectra of highly ionised atoms. The gratings used in these spectrographs were ruled on a ruling engine of Siegbahn's own design.

In 1937 Siegbahn moved to Stockholm to become director of a new research institute created for him by the Academy of Sciences with financial support from the Wallenberg foundation and the State. Established as a department of the Academy's Nobel institute it became generally known as the Nobel Institute for Physics.

Anticipating the coming importance of nuclear physics which began to develop during the first half of the 1930s, Siegbahn planned the new institute especially for research in nuclear physics which up to then had been almost completely neglected in Sweden. The institute was equipped with one of the first cyclotrons in Europe and with excellent facilities for nuclear spectroscopy. For the third time Siegbahn managed to assemble a large group of able collaborators and made the Nobel Institute a centre for nuclear research of high international reputation. Siegbahn formally retired from the directorship in 1964, at the age of 78, but continued to maintain a keen interest in the activities of the Institute.

Manne Siegbahn's success as a research leader was founded on a deep interest in the subject matter as well as a feeling for the potential fertility of a field and an unhesitating readiness to take up new lines of research. As a designer of instruments he saw the essential functional requirements and found the most direct way of solving the problems. In his contacts with people Siegbahn was a man of few words, but what he said was always to the point and his quiet manner was not to be taken as a sign of timidity. On the contrary, he possessed the large amount of contagious self-confidence needed for bold enterprises. Siegbahn took little interest in formal teaching and he gave his research students a great deal of freedom in their work, at the same time keeping them aware of his generous encouragement.

Bengt Edlén

E. A. Moelwyn-Hughes

WITH the death, on 10 September 1978, of Emyr Alun Moelwyn-Hughes, Fellow of Darwin College and for many years Lecturer in the University of Cambridge, the world of chemistry has lost one of its luminaries.

He was born in Cardigan in 1905 one of the six children of the Rev. Dr J. G. Moelwyn-Hughes, a well known Welsh Presbyterian minister and hymnologist. After a remarkable career as a student in the University of Liverpool, who awarded him a DSc at the age of 28, and where his interest in kinetics was first aroused by Prof. W. C. McC. Lewis, he went to Magdalen College, Oxford as 1851 Exhibitioner.

It was there, alongside C. N. Hinshelwood with whom he collaborated, that his work on solution chemistry and the comparison of the rates of change in the gaseous and liquid state first flourished. His interests soon encompassed ionic reactions, enzyme catalysis, the isotope effect and the general theory of solutions. The first of his classical texts *The Kinetics of Reactions in Solutions*, which greatly influenced the subsequent development of the subject, was published in 1933, at about the time when he embarked on a year's collaboration with K. F. Bonhoeffer at the Institut für physikalische Chemie, Frankfurt.

In 1934 he moved to the University of Cambridge where, apart from the war years when he was associated with the Ministry of Supply, he remained for the rest of his life, except for short visits abroad. He held the Messel Fellowship of the Royal Society from 1936 to 1940, and later became a Vice President of the Chemical Society.

As a teacher in Cambridge, and as an extraordinarily successful author of several standard texts, he influenced the careers of countless young scientists. One of his own research associates (J. C. Kendrew) became a Nobel laureate, and many others reached distinguished heights. They were taught at the time when his own experimental contribution was at its apogee: at a time when he wrote the often-quoted 'the complete physical chemist blows his own apparatus and solves his own equations.'

But he will be remembered best for his scholarly authorship of texts which were widely acclaimed. Moelwyn-Hughes wrote about his chemistry with the consummate skill of the accomplished creative artist. His dignified fluency, together with his wit, percipience and perspective, was altogether refreshing in its originality and impact. Molecules, and other inanimate entities of the physical world, were frequently endowed with human qualities; and he contemplated the beauty, magic and mystery of their behaviour in the best traditions of the natural philosopher and poet. He had the innate feeling for the right turn of phrase. On listening to him, or reading his work, one was impressed by the freshness of his metaphors, which were invariably newly minted. And he obviously relished the act of regulating the rhythm of his sentences. His physical chemistry was described with the right words in the right order. Some of the gems that constitute part of his legacy trip off the tongue: 'Energy among molecules is like money among men; the rich are few, the poor numerous.' 'Belief in the essential simplicity of things is one of the chemist's articles of faith.' And consider his discussion of the self-evident fact that the liquid state of matter is intermediate between the solid and gaseous states. 'Like a central party in politics or a moderate denomination in religion, the liquid state is less rigorously defined and more difficult to understand than either of the extremes that flank it.'

His introduction to the Everyman Edition of *The Sceptical Chemist* (1963) is a model of succinct historical exposition. In asking when the discovery of the inverse relationship between pressure and volume was made and who was Robert Boyle, he wrote: 'He flourished in the seventeenth century, that turbulent time of pestilence and fire so amply described by Evelyn and Pepys, when Bunyan wrote in Bedford jail and Penn left England's shores, when Milton sang his *Paradise Lost* and Wren built London's churches, when Britain's monarch was overthrown and Cromwell made Protector.' His book-reviews were also delightfully composed.

Moelwyn-Hughes was greatly admired by many. He was appreciated as a significant figure in twentieth century physical chemistry. His coruscating, if sometimes caustic, wit was much enjoyed (and occasionally feared) as was his superb storytelling gift; and very many including those Welshmen who got to know him at the University of Cambridge Welsh Society (Cymdeithas y Mabinogi), valued his warm-hearted friendship.

His illness was prolonged: for many years he was struck with immobility and incapacity. During that time he uttered not a syllable of complaint; and throughout it all he was nursed with profound affection and care by his devoted wife Mair who, along with his twin sons Edmwnd and Rolant, survive him.

J. M. Thomas

F. C. Fraser

DR FRANCIS CHARLES FRASER, CBE, FRS, Polar Medal 1942, a cetologist of international distinction and Keeper of the Department of Zoology at the British Museum (Natural History) from 1957 to 1964, died on 21 October 1978 at the age of 75. A graduate of Glasgow University, where his interests had been in both zoology and geology, his love of the sea and an appropriate opportunity sent him to the Antarctic and South Georgia from 1925–1933. His early work with the 'Discovery Investigations' was on the development and distribution of the young stages of *Euphausia* (*Discovery Rep.* 1936, 14, 1).

From the time of his appointment to the museum in 1933 as an Assistant Keeper, Fraser devoted his research to various aspects of cetology. He took over the *Reports on Cetacea, stranded on the British Coasts* which had been initiated in 1913 by Sir Sidney Harmer. This involved the development of communications between coastguards and other authorities and the museum, and then recovery, identification, measurement, and preparation of specimens to ensure the fullest scientific use of each stranded cetacean Successive *Reports* were published in 1934, 1946, 1953 and 1974. Each contained analyses of strandings by species, site and distribution, number per month, estimated age, time of birth and summaries of many other findings useful in building up facts about the life histories of cetaceans. Altogether (from 1913–1966) 1,550 cetaceans were identified by Harmer and Fraser. These reports have been so valuable that other countries have begun to publish similar records.

During the Second World War, Fraser worked at the Admiralty. Afterwards he joined the 'Atlantide' expedition (1945–1946) and studied cetaceans off the coasts of West Africa. He soon confirmed his reputation as a leading cetologist, especially on the often difficult decisions of identification: people from all parts of the world came for his help. His numerous taxonomical notes, mostly on rarer species, provided clarification where there was usually utter confusion.

In 1956 he described a species of dolphin *Lagenodelphis hosei* from a single skeleton collected before 1895 on a beach in Sarawak. From 1971 onwards sightings and captures have been made in widely scattered parts of the world to 're-discover' what is now known as Fraser's dolphin

He contributed original observations on the anatomy, especially cranial osteology, and morphology of many cetacean species and made use of stranded animals brought to the museum both for research and display. The Whale Hall's present existence and its popularity owe much to Fraser's desire to educate the public about whales and dolphins He also supported many of the early enterprises to exhibit live dolphins. His informative museum guides to British cetaceans and his splendid book with J R. Norman, *Giant Fishes, Whales and Dolphins* are meticulously accurate

A substantial work was that with P. E. Purves on Hearing in Cetaceans (*Bull. Brit. Mus* (*Nat. Hist.*) *Zool* 1960, 7, 1) This dealt with anatomical and experimental evidence to demonstrate that hearing was by way of the external auditory meatus, it was precisely discriminative and directional and the cetacean ear was sensitive to a wide range of frequencies. The elaborate sinus system was shown to be an extension of the middle ear cavity and could be a guide to the systematic arrangement of the Order Cetacea.

Fraser had a deep interest in the history of cetology and wrote about Peter Mundy's 'Greenwich Whale of 1568, early Japanese whaling, William Scoresby Junior and on the Grey Whale in Icelandic waters. His last contribution (1977) was on 'Royal Fishes: The Importance of the Dolphin'. This paper contained a lifetime's research and reflection on the Royal Prerogative in Britain which he had traced back as far as the 13th century.

Fraser was proud of his Dingwall stock and delighted in being a pawky Scot of the dry, humorous kind; yet there was nothing but solicitude and generosity about him, consideration for anyone deserving of it and an unrelenting determination to see justice done. It could well have been of him that Herman Melville wrote: 'But I have swam through libraries and sailed through oceans, I have had to do with whales with these visible hands; I am in earnest; and I will try.'

R. J. Harrison

A. S. McFarlane

ARTHUR SPROUL McFARLANE, Head of the Biophysics Division at the National Institute for Medical Research from 1945 to 1970 died on 4 October 1978.

He was born in Glasgow on 18 April 1905. He took 1st class honours in biochemistry at Glasgow University in 1928, and qualified in medicine in 1931. From 1931–33 he was biochemist at the Glasgow Royal Cancer Hospital, and was elected to a Beit Memorial Fellowship in 1933 to work in Uppsala under Professor The Svedberg on the use of the recently developed oil-turbine ultracentrifuge. The studies which he made of a wide range of serum proteins illustrated the potential of this new technique for the characterisation of normal and pathological proteins in mixtures.

When the Rockefeller Foundation, anxious to make the instrument available to other laboratories, offered one to the Lister Institute, McFarlane joined the Institute to continue his Beit Fellowship and to supervise the installation of the Svedberg machine in a specially constructed building. The installation was completed in 1937, by which time he had become a member of the Lister Institute staff and had been joined by R. A. Kekwick. They also acquired the newly developed Tiselius electrophoresis apparatus—both instruments being the first of their kind in the UK—and in 1938 published the first paper arising from their use on the "Physical properties of bushy stunt virus" recently crystallised by F C. Bawden and N. W. Pirie.

Further work on vaccinia virus was interrupted by the war, when his attention changed to devising a means for preparing stable human plasma for transfusion. He invented a novel means of removing unstable lipids by freezing below −25 °C with ether, and went on to design large scale freeze drying plant. This was done at the L.C.C. Serum Institute, Carshalton, where the laboratory had been transferred in 1941.

In 1943 McFarlane's services were requested by the National Institute for Medical Research to supervise the design and installation of special services, including one of the early electron microscopes, in the new building at Mill Hill (built in 1938 but used meanwhile by units of the Women's Royal Naval Service and finally occupied in 1950).

When in 1945 the US Atomic Energy Commission announced that radioisotopes produced in uranium chain-reactors would be available for peaceful uses to scientists all over the world, McFarlane was sent by the Medical Research Council to negotiate the supply of radioisotopes for the UK. Hopes were rudely shattered by the passage in 1946 of the McMahon Act forbidding exportation from the US of all radioactive materials produced in atomic piles, but McFarlane through his friendship with Robley Evans, in charge of the cyclotron at the Massachussetts Institute of Technology, managed to obtain sufficient ^{32}P to allow research workers here to get the feel of radioisotopes and experience in design of counting instruments.

In 1947 he was again sent to the US and to Canada to negotiate purchase of other radioisotopes and this mission was entirely successful. (A fuller account appears in an article by Professor George Popjak in *Trends in Biochemical Sciences*, October 1976.)

By the time that the N.I.M.R. finally moved to Mill Hill in 1950 McFarlane had been responsible for making it the best equipped medical research laboratory in England in respect of biophysical instrumentation. By designing an elaborate but efficient apparatus for counting ^{14}C after combustion to ^{14}CO$_2$ and installing a mass spectrometer he also gave his colleagues a headstart in the use of the radioactive and stable isotopes then available.

His own interests became concentrated on studying the metabolism of plasma proteins, using ^{14}C and radioiodine as trace labels. He devised a method for labelling proteins with radioiodine, which avoided denaturation and by studies on antibodies in collaboration with J. H. Humphrey showed that biological activity and ^{14}C internal and ^{131}I external labels were catabolised at identical rates. This validated radioiodine labelling and led to a series of definitive measurements on the turnover of several plasma proteins in normal and sick subjects, in which McFarlane collaborated with workers in Britain, the USA and elsewhere. He also developed an ingenious method for measuring plasma protein synthesis *in vivo* involving simultaneous measurement of incorporation of ^{14}C from labelled urea and ^{14}C from bicarbonate.

By temperament he was a shrewd Scotsman, with what might nowadays be considered an old fashioned outlook on life, but certainly capable of enjoying it. He was at his best when devising apparatus or techniques, and would have made his mark in industry had he chosen this career. Although his own approach to biological problems was simple (sometimes over-simple) and mathematical, he greatly helped many biological colleagues by providing them with technological back-up at a time when the array of sophisticated equipment nowadays provided by scientific instrument manufacturers was not available.

J. H. Humphrey

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newly on the market

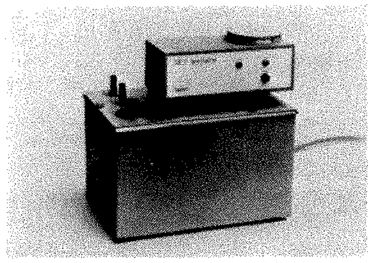
Nitrate electrode. Electronic Instruments. A nitrate electrode has been added to the range of ion-selective electrodes of Electronic Instruments. Model 8006-2 is of the capsule design ensuring easy assembly and minimal maintenance. The new ion exchanger used gives improved selectivity for NO$_3$ in the presence of Cl$^-$, an important breakthrough in nitrate determinations in sewage and industrial effluents.
Circle No. 47 on Reader Enquiry Card.

Nuclear magnetic resonance spectrometer. Perkin-Elmer. Perkin-Elmer announces the model R-600 high-resolution, Fourier Transform NMR spectrometer. A high stability permanent magnet provides a 14,092 gauss field for proton observation at 60 MHz. Combined with an easy-to-use microcomputer data system to control instrument functions, the R-600 offers users the capability of obtaining NMR spectra, in a routine manner, on quantities of 500 mg or less, at half the cost of existing Fourier Transform instruments. High resolution spectra over long-term accumulation are possible with the model R-600 through the use of an internal deuterium field/frequency lock that provides a field stability within a few tenths of a Hertz. System resolution is 0.5 Hz. Integration accuracy is 2.5% (5% ethyl benzene).
Circle No. 48 on Reader Enquiry Card.

Regulator. Tescom. Tescom 44-2200 series regulators are designed to prevent contamination of high purity systems and provide accurate regulation of any corrosive or noncorrosive gas or liquid. For corrostive applications, all media wetted parts are type 316 stainless steel or Du Pont Teflon. High purity systems are further protected from contamination through metal-to-metal sealing of diaphragm between body and bonnet. Soft seals are not used. Models are available for primary pressures of 3,000 p.s.i.g. and 400 p.s.i.g. with choice of secondary pressure ranges of 0–25 p.s.i.g., 0–50 p.s.i.g. and 0–100 p.s.i.g. Gauge ports standard on cylinder regulators.
Circle No. 46 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

Constant temperature circulator. Techne. In response to the frequent need in the laboratory for a small low-cost compact constant temperature circulator, Techne has introduced the Model SP670 open tank circulator. This unit was developed specifically to provide a controlled temperature of 55 °C in the reaction column of a solid phase peptide sequencer. However, the unit will also find general use in the laboratory. The model SP-670 uses the TE-7 solid state immersion circulator with 500 W heater fitted to a small deep drawn tank and with inlet and outlet pipes for connection to a closed circuit external system. The bath is fitted with a top up port. Temperature range is 0–95 °C and control obtainable is ±0.02 °C.
Circle No. 45 on Reader Enquiry Card.



The TE-7 immersion circulator

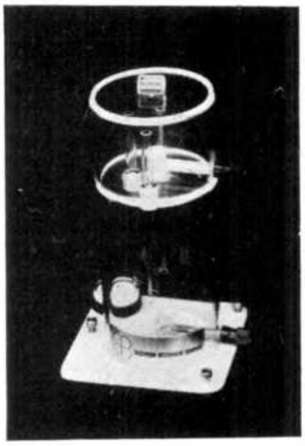
Four-channel non-fade scope. British American Optical. The model 1144, a four-channel non-fade scope, provides monitoring of ECG, arterial pressure, plus third and fourth parameters on a large, bright, moving trace display. Internal selections permit adaptation of the display format to many different monitoring situations. Circuitry includes four dynamic shift register memories each with 1,024 word capacity, delayed outputs being coded to facilitate distinguishing between real time and delayed signals, and between those that were previously frozen and those that were not.
Circle No. 44 on Reader Enquiry Card.

Effluent quantity and quality meter. Philips. A new portable flow meter from Philips can carry out the accurate measurement of waste water flow rates automatically, even in the presence of surface foam, scum or floating objects. The PW 9816 meter uses a dipping probe to sense the level of water in a measuring channel of known cross section, giving a direct correlation with the rate of flow. Attached to a weighted flexible cable mounted on a rotating drum, the probe is raised and lowered at regular intervals. Contact with the water surface completes an electrical circuit. The resultant signal is electronically converted and used to drive a pen that produces a trace on a circular chart. The PW 9816 can be used in conjunction with a wide range of standard wiers, flumes and venturis, and produces a record of flow rates in m^3 min^{-1} and, via a computing integrator, total flow since the start of measurement.
Circle No. 42 on Reader Enquiry Card.

IBM—compatible terminal system. Control Data announce the first of a series of multiple-function computer terminals that can be operated as stand-alone processors to terminal devices by business users of IBM 360 and 370 computer systems. The unit, called the Control Data C760 multifunction terminal, can be programmed by non-technical operators. The clerical operator can generate and execute application programs on the terminal using a simple forms language that is provided with the basic terminal system. The CDC C760 terminal is available in a minimum configuration that includes a microprocessor-controlled keyboard and visual display terminal.
Circle No. 43 on Reader Enquiry Card.

Environmental monitor. Research Products International. Now available from Research Products International Corporation is a portable G-M tube environmental monitor, Model 6-70. Supplied with a special compensated MC-70 type G-M tube, this instrument is designed for determining environmental γ radiation levels. The highly sensitive detector is suitable for measuring low intensity radiation within a reasonable time period. Sensitivity is approximately 150 pulses s^{-1} for a 1 μR h^{-1} exposure rate. Measurement over a period of 1,000 s will give a result having a statistical accuracy of ±3% for a background of 7 μR h^{-1}. The Model 6-70 is designed for field use and is powered by Ni-Cd rechargeable batteries giving 10 h run per charge. A tripod stand is available allowing the detector to be located 10 m away from the instrument.
Circle No. 50 on Reader Enquiry Card.

Tube gel electrophoresis apparatus. Dickinson. This three-sample, tube gel electrophoresis apparatus from Dickinson is designed for quick tests of molecular weight range, restriction enzyme activity, buffers, ampholytes and gradients. The model TG-3 accommodates all types of gel techniques including polyacrylamide, agarose and isoelectric focusing. Up to three, 5 mm diameter tube gels with a length between 4 cm and 15 cm can be run simultaneously. The apparatus has clear acrylic plastic construction and platinum wire electrodes.
Circle No. 51 on Reader Enquiry Card.



The Dickinson model TG-3

Emission spectrometer. Philips. Modular construction and the availability of a wide range of sample excitation options make the new PV 2850 emission spectrometer from Philips an adaptable system for fast, simultaneous multi-element analysis of solids and liquids. This compact air-path spectrometer operates automatically and can be used to determine up to 60 elements simultaneously. A choice of modular electronics can be incorporated, while various computer hardware/software packages are available for instrument control and output of results as element concentrations.
Circle No. 52 on Reader Enquiry Card.

Cold light illumination system. Specfield. A fibre optic cold light illumination system, the SLS 75 light source with double-branched flex-and-stay fibre bundle, has been announced by Specfield. Primarily designed for microscope illumination and as a cold-light source for close-up photography of small heat-sensitive subjects, the SLS 75 houses a 75-W fan-cooled quartz iodine lamp. The light from the lamp is concentrated onto the end of a double-branched fibre-optic light guide which is securely held in the front panel of the unit. The unit operates on 240 V a.c., and a 110 V a.c. version is available.
Circle No. 53 on Reader Enquiry Card.

Pressure filtration apparatus. Millipore. Millipore's Teflon pressure filtration apparatus is made entirely of non-metallic surfaces and is designed for field or laboratory use in sampling all types of waters where there is a requirement to separate particulate from dissolved heavy metals fractions. Certified filters of specified metallic ion content are used to retain the particulate fraction. These can then be totally digested in suitable acid cocktails for subsequent amino acid or other instrumental analysis to determine and differentiate particulate from dissolved heavy metals. Filtering of 500 ml–6 l of potable and highly turbid waters is effected in a matter of minutes.
Circle No. 54 on Reader Enquiry Card.

Gel scanner. ISCO. The ISCO model 3010 gel scanner will produce both UV scans on unstained gels and visible scans on stained gels. UV scanning of a cylindrical gel in its original quartz running tube simplifies determination of the minimum time required for the maximum revolutions and provides accurate quantitation of each peak. The unit consists of the ISCO absorbance chromatography monitor, model UA5 and the gel transporter unit, thereby offering an instrument for performing two functions in the protein laboratory.
Circle No. 55 on Reader Enquiry Card.

Medical linear accelerator. Varian. The Clinac 20 from Varian is a highly versatile dual-modality medical linear accelerator that offers great flexibility in treating malignant tumours, and can selectively generate either a 15 MV X-ray (photon) beam or an electron beam variable over 6–20 MeV. The Clinac 20 has an output of up to 500 rads per min with treatment field sizes that range from 0×0 to 35×35 cm at the 100-cm isocentre. The extra high output means treatment can be given to patients in less time than is now required with lower radiotherapy machines.
Circle No. 56 on Reader Enquiry Card.

Electrophoresis power supply. Buchler. The model 3-1500 constant power supply from Buchler Instruments offers researchers the convenience of a bright digital display of output with the exacting performance required for such procedures as electrofocusing. Push-button controls select the appropriate output ranges and regulation mode. Constant voltage, constant current and constant power may be selected at levels up to 1,000 V d.c. 200 mA and 200 W. A fault circuit prevents any damage from occurring to the unit in cases of external shorts or open circuits.
Circle No. 57 on Reader Enquiry Card.

Digital thermometer. Channel Electronics. Designed for bench or rack mounted applications, the 7500 series of mains powered digital thermometers have been introduced by Channel Electronics. Nine basic units are available, with thermocouple and platinum resistance inputs, and together they offer a temperature measurement capability from −220 °C up to 1,750 °C, to a resolution of either 0.1 °C or 1.0 °C. Thermocouple units incorporate an improved cold junction compensation system for greater stability and reliability. The gas discharge display provides a clearly discernible read-out at a range of up to 40 ft. Options include a fully linearised voltage output and non-standard calibrations are available for all linear or non-linear millivolt, milliamp or resistance inputs.
Circle No. 58 on Reader Enquiry Card.

Laboratory titrators. Electronic Instruments Limited. A major expansion of the EIL laboratory product range has taken place with the launch of a comprehensive series of automatic laboratory titrators. The range, consisting of Karl Fischer, endpoint, *ph* stat and recording titrators, all incorporate a unique photoelectric counting device, which ensures that titrant and samples are dispensed with the highest possible precision.
Circle No. 59 on Reader Enquiry Card.

EIL Laboratory titrators

Stereoscopic microscopes. Olympus. The Series VM of stereoscopic microscopes from Olympus consists of two basic models, VMT and VMF. The VMT is a turret magnification change type with ×1 and ×2 or ×1 and ×4. The VMF is a fixed magnification model with choice of ×1, ×2 or ×4. All versions share a common range of auxiliary lenses, eyepieces, stands and accessories. Special attention has been paid to the lighting available with the series and the stand has been designed to accept a minimum of 11 different methods of illumination. A very special feature is the base illuminator which offers transmitted, epi' or combined illumination.
Circle No. 60 on Reader Enquiry Card.

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UNIVERSITY OF ASTON IN BIRMINGHAM

DEPARTMENT OF BIOLOGICAL SCIENCES

FISH NUTRITIONIST

Applications are invited for a Lectureship in Biological Sciences. The successful candidate will be expected to contribute to the teaching of general nutrition and metabolic biology within the Department. Candidates must have research interests in fish nutrition, preferably in the field of vegetable and single cell protein utilisation

Commencing salary will be within the range £3,883 to £5,842 per annum on a scale rising by annual increments to £7,754 per annum (under review).

Application forms and further particulars may be obtained from the Staff Officer (Ref. No 331/6) University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (Tel 021 359 3611, Ext 201) to whom applications should be forwarded not later than February 23, 1979

9287(A)

UNIVERSITY COLLEGE LONDON

LECTURER IN CHEMISTRY

Applications are invited for a Lectureship in the Department of Chemistry from October 1, 1979 Candidates should have special interests in organic chemistry or in biological chemistry or in both It is expected that the appointment will be made within the lower part of the lecturer salary range, the whole of which is £3,909 to £7,754 plus £450 London Allowance.

Further particulars can be obtained from the Assistant Secretary (Personnel), University College London, Gower St, London WC1E 6BT, and applications (6 copies if possible) should reach him on or before March 16, 1979

9330(A)

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research

IMMUNOLOGIST

A young postdoctoral immunologist is required by the LABORATORY FOR LEPROSY AND MYCOBACTERIAL RESEARCH to investigate immunity of mycobacterial infections. It is proposed that the person appointed will study particularly the immunological control of the growth of mycobacteria in tissue culture and in animals

The appointment will be for up to three years and will be in the salary range £4,408 to £6,080 per annum according to age and qualifications, plus £450 p a. London Allowance Superannuation provision

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA, before March 31, 1979.

9334(A)

EXPERIENCED RESEARCH ASSISTANT

required for a one-year project in human metabolism. Work involves amino acid analyses and metabolite assays as part of an investigation of parenteral nutrition.

Salary c.a £3,930 depending on qualifications and experience.

Apply in writing to Dr B. D Ross, Department of Clinical Biochemistry, Radcliffe Infirmary, Oxford. 9307(A)

DIRECTOR DALTON RESEARCH CENTER UNIVERSITY OF MISSOURI COLUMBIA

This multidisciplinary facility for basic biological research, graduate and postgraduate training is supported principally from state funds, as well as from Federal agencies and foundations. A new building of 60,000 sq ft in Research Park, adjacent to the main campus, has excellent research laboratories with modern animal shop, computer, controlled environment, and other facilities

A Director is sought with commitment to outstanding research who will develop a dynamic research unit in a life science area. The Director will be expected to bridge and complement Dalton Center activities with selected existing talents and resources on the UMC campus, examples are cardiovascular regulation, cellular and developmental biology, molecular genetics, immunobiology, neurosciences, and bioenginering.

This is intended as a "Center of Excellence" and the University is committed to providing a competitive Director's salary and other resources to insure success. The Director should have research management experience, including budgeting, recruiting, and generating research funds.

The Director will be tenured in an academic department of UMC The University is a Land Grant school with a student enrollment of 23,000 On a single campus, it has a unique combination of life science-oriented Divisions (Medicine and other health related sciences, Agriculture, Veterinary Medicine, Engineering, and Biological Sciences) Particular research strengths may be found in departments of Biochemistry, Physiology, Biological Sciences, Bioengineering, Medicine, Microbiology, Pharmacology, Radiology, and Surgery University resources include Reactor Research Center, Cancer Research Center, Health Services Research Center, Veterans Administration Hospital, University of Missouri Medical Center, and Eye Research Institute

Women and members of minority groups are urged to apply Résumés and other appropriate information should be sent to:

Search Committee for Director
Dalton Research Center
Research Park
University of Missouri
Columbia, Missouri 65211

Closing date: March 10, 1979.

The University of Missouri is an Equal Opportunity Employer

9335(A)

APPOINTMENTS VACANT

CHAIRPERSON

BIOCHEMISTRY DEPARTMENT

THE UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL

is seeking an individual who has a proven commitment to excellence in research and has demonstrated expertise in teaching and administration to chair its Department of Biochemistry The Department presently has strength in the areas of cyclic nucleotides, hormonal control of metabolism and nutrition. Several faculty positions will become vacant by September of 1979 and may be filled by the new chairperson. The successful candidate will be expected to lead a department that is both exceptional in its research and committed to quality teaching of biochemistry to medical and graduate students

Please send curriculum vitae, bibliography and three references by March 15, 1979 to: Dr F. S Fay, Chairman, Search Committee, Department of Physiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01605.

Minority and women candidates are encouraged to apply The University of Massachusetts is an equal opportunity/affirmative action employer

9339(A)

THE UNIVERSITY OF LEEDS

PROCTER DEPARTMENT OF FOOD SCIENCE

RESEARCH FOR SCHOOL SCIENCE MASTERS

Applications are invited from graduate Science Masters in Schools to take part in research in the Procter Department of Food Science for a period of four weeks in the summer vacation. Two places are available in 1979 and grants to cover expenses incurred are available from the Worshipful Company of Skinners through its Lawrence Atwell's Charity. The research will be of a chemical or physical nature and topics will be selected with subsequent continuation in school laboratories in mind either as part of the school science syllabus or as a research project. The scheme is subject to an age limit of 29.

Applications, giving details of career and interests, and the names of two referees, should be sent as soon as possible to the Head of the Department, Proctor Department of Food Science, University of Leeds, Leeds LS2 9JT.

9297(A)

WESTMINSTER MEDICAL SCHOOL DEPARTMENT OF THERAPEUTICS

requires a

Research Assistant

to join an active research team, including medical staff of Westminster Hospital, who are studying the clinical pharmacology of drugs used in the chemotherapy of cancer.

Candidates should be graduates in chemistry or a biological discipline. They should also have a sound practical knowledge of the techniques of drug analysis, including G.L.C. and H.P.L.C., coupled with some research experience. The successful applicant will also participate in the on-going departmental research programme relating to molecular mechanisms of drug action.

Salary, according to age and qualifications, will be in the range £3,834 to £5,332, inclusive of London Weighting (review pending).

Applications, with names and addresses of two referees, to Dr B. A. Brooks, Department of Therapeutics, Westminster Hospital, London SW1P 2AP. Tel: (01) 828-9811 ext. 2490.

9316(A)

Science v. Crime

Opportunities for graduates in Advanced Forensic Investigation.

In the fight against crime science is an increasingly active agent in uncovering facts from subtle but often decisive evidence in cases such as murder, rape, arson and burglary. Science can successfully answer the critical questions—"who?", "when?", "where?", "how?"—which provide the police and the courts with invaluable assistance in the maintenance of law and order and the administration of justice.

The Forensic Scientists in the Home Office have access to advanced analytical techniques which bring to light new sources of evidence. The forensic laboratories at Aldermaston, Birmingham, Bristol, Cardiff, Chorley, Nottingham and Wetherby (the laboratories at Bristol and Cardiff will move to Chepstow in 1979) are being comprehensively equipped with sophisticated instrumentation for use in gas and high pressure liquid chromatography, mass spectrometry, scanning electron microscopy, electron microprobe analysis, radio immuno assay, atomic absorption spectrophotometry, X-ray fluorescence spectroscopy and other methods.

Work in the Forensic Science Service offers graduates opportunities for advanced scientific investigation associated directly with criminal investigation—often involving visits to the scene of the crime and giving evidence at court.

There are openings for honours graduates (including those qualifying in 1979) in chemistry, biochemistry, biology, pharmacy, physics, materials science and related scientific disciplines, and encouragement will be given to obtain further qualifications. Appointment as Scientific Officer (£2,840 to £4,410) or Higher Scientific Officer (£4,100 to £5,450), according to qualifications and experience. Good colour vision is essential.

For further details and an application form (to be returned by February 19, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours).

Please quote ref: SY/22/LA/2.

9347(A)

UNIVERSITY OF WALES

UNIVERSITY COLLEGE OF SWANSEA

SENIOR RESEARCH ASSISTANT

Applications are invited from physicists/electrical engineers with experience in Computing, or from mathematicians/computer scientists, with an interest in the application of numerical methods to physical situations, for the vacancy of **Senior Research Assistant in the Department of Physics.** The successful applicant will work on an S.R.C. sponsored project to simulate numerically the electrical breakdown of a gaseous dielectric.

The appointment which will be for two years from the soonest date that can be arranged, will be on a scale up to £4,382 per annum plus U.S.S./U.S.D.P.S. benefits.

Further particulars and application forms (two copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by Friday, February 9, 1979.

9301(A)

GLASGOW COLLEGE OF TECHNOLOGY

The Glasgow College of Technology, a major polytechnic institution of higher education, invites applications for the following post:

RESEARCH ASSISTANT in OPHTHALMIC OPTICS

The successful applicant would be required to work on an agreed topic in Ophthalmic Optics. Applicants should hold appropriate qualifications in Ophthalmic Optics, or alternatively an honours degree in a discipline such as Physics, physiology or psychology, and should have a strong interest in visual problems. The person appointed will be expected to read for a higher degree of the CNAA, Master or Doctor of Philosophy.

Salary: The Research Assistant will be paid 50 per cent of the appropriate point on the Lecturer 'A' scale: £4,029 to £7,110 Bar-£7,638. Placing on the salary scale will be according to relevant experience.

Application forms and further particulars can be obtained from the Establishments Officer, Cowcaddens Road, Glasgow G4 0BA.

Application forms should be returned not later than Friday, February 2, 1979.

Edward Miller, Director of Education, Strathclyde Regional Council.

9267(A)

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

RESEARCH ASSISTANT
IN BONE AND JOINT UNIT

Applications are invited for the post of Research Assistant in the Bone and Joint Unit. The post will be tenable for approximately 2½ years.

The project with which the successful applicant will be associated concerns development and evaluation of methods of detecting autoantibodies in human disease.

Candidates should have an I.M.L.S. qualification or appropriate practical experience in medical laboratory work.

Salary: £3,384 to £4,882 plus £450 London Allowance per annum, together with superannuation under the U.S.S.

Enquiries to Professor E. J. Holborow. Telephone: 01-247 8939.

Applications to the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, enclosing curriculum vitae and names of two references.

9300(A)

POSTDOCTORAL POSITION

open for chemist/biochemist/crystallographer interested in protein and nucleic acid crystallisation.

Starting March 1: DM1,900 per month tax free.

Send C.V. and list of publications to Prof. Dr W. Saenger, Max-Planck-Institut für experimentelle Medizin, Hermann-Rein-Str. 3, 3400 Göttingen, **W.-Germany.**

9299(A)

MEDICAL RESEARCH COUNCIL
DUNN NUTRITION UNIT

Postdoctoral research worker required to undertake studies on energy balance in man. Studies will be conducted in a new metabolic suite incorporating three whole body calorimeters at the Unit's Clinical Nutrition Centre, Addenbrooke's Hospital, Cambridge. Experience in human physiology desirable, with or without a medical qualification. The appointment is for three to five years, with salary according to qualifications.

Further details and applications by February 15, 1979, to Dr W. P. T. James, Dunn Clinical Nutrition Centre, Addenbrooke's Hospital, Trumpington Street, Cambridge CB2 1QE.

9186(A)

COMMUNITY ECOLOGIST

The University of Kansas anticipates a 12-month tenure-track position as Assistant Professor, Division of Biological Sciences, and Assistant Curator, Museum of Natural History (joint appointment) available July 1, 1979. Community Ecologist with theoretical emphasis; experience with vertebrate communities and their vegetational bases. Duties curatorial and teaching, including research and graduate student guidance. Ph.D. required prior to assumption of duties. Send curriculum vitae, reprints, and direct three letters of reference to: Frank B. Cross, Museum of Natural History, University of Kansas, Lawrence, KS 66045, by March 15, 1979. Salary range $17,000 to $19,000. Equal Opportunity/Affirmative Action Employer.

9284(A)

UNIVERSITY OF LONDON
ROYAL POSTGRADUATE MEDICAL SCHOOL
SAFETY OFFICER

Applications are invited for this newly established post, the holder of which will be Secretary of the Safety Committee and will be responsible to the School Secretary for the implementation of safety policy.

Applicants should have a sound knowledge of safety matters. Particular knowledge of safety considerations in laboratories would be an advantage, but is not essential.

Salary on grade II for academic-related Administrative Staff: £6,317 to £7,754 (under review) plus £450 a year London Allowance.

Superannuation under Universities Superannuation Scheme.

Further particulars should be obtained from the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS (tel. no: 01-743 2030 ext 352), to whom applications (6 copies) should be sent, naming three professional referees, to arrive not later than Wednesday, February 28, 1979. 9253(A)

CHELSEA COLLEGE
(University of London)
BASIC MEDICAL SCIENCES GROUP

There is a vacancy in the Physiology Department for a

RESEARCH AND TEACHING TECHNICIAN (Grade 4)

with an interest in practical electronics applied to electrophysiological problems We should be pleased to hear from suitably qualified or experienced and interested persons who should, in the first instance, contact Susan Holly, Department of Physiology, Chelsea College, Manresa Road, London, SW3 6LX (01-352 6421) for further details and application forms

The salary scale for this post is £3,420 to £3,867 per annum (under review), including £465 London Allowance. 9252(A)

UNIVERSITY OF LONDON
INSTITUTE OF PSYCHIATRY
RESEARCH WORKER
(Scale 1A)

required for a investigation of factors controlling the enzymic methylation of tryptamines in animal and human tissues. Supported by MRC grant

Salary in range £3,384 to £4,382 per annum plus £450 London Allowance. Application forms and further details available from The Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF quoting reference RR/N Application forms should be returned by February 23, 1979 9251(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL
DEPARTMENT OF CLINICAL PHARMACOLOGY
SCIENTIFIC OFFICER

equired to work on a number of reearch projects, centred on hypertenion The projects will include radiodgand binding studies of receptors, rostacyclin pharmacology and catebolamine turnover The candidate hould possess a degree or equivalent n a related subject Experience in eneral biochemical techniques is required and a working knowledge of at east one of the projects is desired

Salary on scale £3,887 to £6,169 a ear, starting salary dependent on exerience.

Application form and further partiulars may be obtained from the Peronnel Office, Royal Postgraduate edical School, 150 Du Cane Road, ondon W12 0HS quoting reference umber 20/103/N 9250(A)

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unilever research

Ice Cream Research Assistant

Our Ice Cream and Dairy Section has a vacancy for a Research Assistant.

Tasks:

The job will involve:

(i) obtaining physical test data on ice cream and relating this to sensory properties, formulation etc;
(ii) the organization of the running of the ice cream mini-line;
(iii) work on development of new products generally in connection with Unilever companies.

It will also involve organising the work pattern of an assistant and trying to develop a detailed understanding of the structure and important consumer attributes of ice cream. The job will require an appreciation of the different requirements for ice creams in various countries and an ability to identify and analyse problems with equipment under the supervision of the job holder.

Qualifications:

Candidates of either sex, should have a sound scientific background. He or she should have an interest in the measurement of both sensory and instrumental properties of foods as well as an interest in food processing and product development. The position would be suitable for a graduate with a physical sciences degree and little or no experience although experience of ice cream would be an advantage.

Colworth Laboratory

The Colworth Laboratory is located in a very attractive 1200 acre country estate ten miles north of Bedford. Salaries and fringe benefits are at a level expected of a large international company and includes assistance with moving expenses where appropriate.

Please write quoting reference CH-491A and giving details of career history to:

Miss J. Kenny,
Recruitment and Career Development Manager,
Unilever Research,
Colworth Laboratory,
Unilever UK
Central Resources Ltd.,
Colworth House, Sharnbrook,
Bedford, MK44 1LQ.

9315(A)

UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL
RESEARCH MEDICAL LABORATORY SCIENTIFIC OFFICER

required for three years to join a clinically based research team led by Professor A N Exton-Smith, Barlow Professor of Geriatric Medicine, studying the Immunology of Dementia

Starting salary £3,582 and London Weighting on incremental scale

The work will involve the application of immunological techniques to samples from normal and demented aged people. Preference will be given to candidates with some experience in laboratory immunology

Applications and enquires to Dr R Callard, Human Immunology Laboratory, University College Hospital Medical School Tel. 01-387 9300, ext 199 University Street, London WC1 9342(A)

UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL
(University of London)
DEPARTMENT OF GASTROENTEROLOGY
GRADUATE OR RESEARCH TECHNICIAN

Applications are invited for the above post to develop new methods of pancreatic function A sound practical knowledge of biochemistry is essential and experience in radioimmunoassay though not essential would be desirable. The post is for 2 years in the first instance and the salary, within the range £3,261 to £4,680 (plus London Weighting).

For further details please telephone Dr P R Salmon, 01-388 2411, ext 241. Applications giving details to The Secretary, University College Hospital Medical School, University Street, LONDON WC1E 6JJ, quoting Reference RT/Ga 9343(A)

UNIVERSITY OF LONDON
Institute of Psychiatry
DEPARTMENT OF NEUROPATHOLOGY
JUNIOR TECHNICIAN

required to work on project involving research on biochemical causes of brain damage Basic knowledge of methods of biochemical analysis, especially of amino acids, desirable and an interest in electronics an advantage Applications from recent school leavers with 'A' level Chemistry would also be considered.

Whitley Council Terms and Conditions for Medical Laboratory Junior Scientific Officers A or B. Starting salary in range £2,259 to £3,243 per annum (including London Weighting) depending on age and qualifications. The post is grant-supported for three years.

For application form, please write to the Assistant Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF or Telephone 703 5411, Ext 214, quoting reference OEP/N. 9291(A)

Wellcome

Biochemist/Molecular Biologist

£5,000 – £9,000

Department of Immunochemistry

A postdoctoral scientist with proven experience in techniques leading to the identification, isolation and characterisation of specific DNA sequences is required to join an enthusiastic young group pursuing the study of cell surface antigens. It is anticipated that the person appointed will be anxious to involve others in the application of DNA technology to the identified research objectives.

Candidates should have a relaxed attitude to collaboration and the motivation and ability to make a significant contribution to a basic research programme with the long-term objectives of better understanding, diagnosis and prevention of disease.

Starting salary will be in the range £5,000-£9,000 per annum and will be dependent on age and experience.

The Laboratories are situated in pleasant parkland surroundings within easy reach of Central London and conditions of employment are excellent and include subsidised canteen, pension scheme, 4 weeks' holiday, relocation expenses where appropriate and sports and social facilities.

For an application form, please write to the

Personnel Manager,
The Wellcome Research Laboratories,
Langley Court, Beckenham, Kent BR3 3BS.

9326(A)

REGIONAL CYTOGENETICS LABORATORY, EAST BIRMINGHAM HOSPITAL

BASIC GRADE SCIENTIFIC OFFICER

Applications are invited for the new post of Scientific Officer. Experience in cytogenetics desirable but not essential Salary range depending on experience and qualifications—£2,991 to £4,899 per annum.

Application forms and job descriptions obtainable from Regional Cytogenetics Laboratory, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST Tel. No. 021-772 4311, Ext. 4354. 9318(A)

UNIVERSITY OF GLASGOW

SECOND CHAIR OF ZOOLOGY

The University invites applications for a second chair in its Department of Zoology.

Further particulars may be obtained from the Secretary of the University Court, (Room 18), University of Glasgow, Glasgow G12 8QQ, with whom applications (15 copies, 1 copy in the case of overseas applicants), giving the names and addresses of three referees, should be lodged on or before March 16, 1979

In reply please quote Ref. No. 4270M 9317(A)

THE UNIVERSITY OF HULL

DEPARTMENT OF PLANT BIOLOGY

GRADE 6 TECHNICIAN

Applications are invited for the post of Technician in charge of the teaching services in the Department of Plant Biology. Candidates should hold an H.N.C. or equivalent qualification and have 9 to 10 years experience. The post is tenable from mid-April Salary on the scale £3,654 to £4,365 (under review).

Applications, giving details of age, qualifications, experience and the names of two referees should reach the Personnel Officer, University of Hull HU6 7RX, by February 9, 1979 Further particulars are available

9324(A)

MEDICAL RESEARCH COUNCIL

PNEUMOCONIOSIS UNIT

Llandough Hospital, Penarth, South Glamorgan CF6 1XW

Young graduate or equivalent required to work on the development of new physical methods for quantifying asbestos fibres in human tissues and in the air Some experience of instrumentation would be helpful.

Starting salary dependent on age and experience but probably within the range £3,261 to £3,582.

Application forms obtainable from the Assistant Administrative Officer at the above address. 9322(A)

UNIVERSITY OF TORONTO

Physiology/Biochemistry

Applications are invited for two research appointments in the general area of gastrointestinal physiology, anaerobiasis and carbohydrate/protein intermediary metabolism

(a) Professional Associate Appropriate doctorate and postdoctoral experience required Initial appointment up to 5 years, renewable. Salary scale floor $16,000.

(b) Postdoctoral Fellow Two-year appointment Initial Salary in range $11,000 to $12,000.

Applications and the names of two referees to:

Professor D. F Mettrick,
Chairman,
Department of Zoology,
University of Toronto,
Toronto, M5S 1A1, Canada.

9327(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF GEOLOGY

LECTURESHIP IN PALAEONTOLOGY

Applications are invited for the post of Lecturer in Palaeontology in the Department of Geology.

Salary according to qualifications and experience on the scale £3,883 to £7,754 a year (under review) with superannuation benefits.

Further particulars from the Registrar to whom application should be sent by February 15, 1979. 9311(A)

UNIVERSITY OF QUEENSLAND

Australia

CHAIR OF MEDICAL BIOCHEMISTRY

The University invites applications for the Chair of Medical Biochemistry to replace Professor Chev Kidson who has accepted the position of Director of the Queensland Institute of Medical Research. Applicants should have a medical degree acceptable for registration within Queensland, and an appropriate higher degree or higher clinical qualifications. They should present evidence of achievement in biochemical research, and should have substantial teaching experience. Applications will be considered from distinguished medical biochemists who do not have medical qualifications but such applicants would be required to present evidence of significant clinical involvement in previous employment.

The successful applicant will play an important role in the development of innovative courses in basic and applied biochemistry for medical students.

The present salary for a Professor is $A33,061 per annum, plus a clinical loading, in the case of a medically qualified appointee, of up to $A5,000 per annum. The University provides prescribed travelling and removal expenses, superannuation, study leave (under review) and housing assistance. Applications close on **April 30, 1979.**

SENIOR TUTOR IN PHYSIOLOGY

Applicants should have an Honours, Masters or equivalent degree preferably in Physiology and will be expected to assist in organising practical classes in this subject and to take part in the Departmental research Programme. This currently includes work on endocrinology, renal or reproductive physiology, fetal or developmental physiology. Closing date February 10, 1979. Salary· $A13,606 to $A15,586 Other Benefits: Superannuation, Housing Assistance, travelling and removal expenses.

Additional information and application forms for both posts are obtainable from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. 9292(A)

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PAEDIATRICS

Applications are invited for the post of TECHNICIAN (Grade 3) to assist with investigations into the physiology of somatomedins and the hormonal control of foetal growth. The work will involve the incubation of animal tissues and applicants should possess qualifications in the biological sciences Salary on scale £2,688 to £3,060 per annum (under review) Applications together with a curriculum vitae and the names of two referees should be sent to Dr D. J Hill, Department of Paediatrics, the Children's Hospital, Sheffield S10 2TH. 9319(A)

TWO POSTDOCTORAL POSITIONS

Study of nucleoskeleton from cultured animal cells and its role in the supramolecular structure of the nucleus and metaphase chromosomes Experience with DNA, chromatin, etc. desirable Study of structure and biosynthesis of cell surface heparan sulfate and possible involvement in cell interactions and with cytoskeleton. Experience in carbohydrate biochemistry preferred. Ph D in Biochemistry, Cell Biology, or allied field $12,000 plus per year Send résumé and two recommendation letters to John M. Keller, Ph D., Department of Biochemistry, University of Health Sciences/The Chicago Medical School, 2020 West Ogden Avenue, Chicago, IL 60612. Affirmative Action/Equal Opportunity Employer. 9328(A)

Institute of Cancer Research—Laboratories at Sutton, Surrey. RESEARCH BIOLOGIST required to join laboratory group investigating biology of human leukaemia; laboratory experience in tissue culture essential. The appointment is for a three year period initially. Salary according to experience in range £3,261 to £4,690 per annum. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/54.
9256(A)

UNIVERSITY OF KEELE
Second Chair in Department of Biological Sciences

Applications are invited for the new post of Professor in the Department of Biological Sciences tenable from October 1, 1979. Applicants may be from any biological discipline except biochemistry. Salary in professorial range plus membership of F S S U / U S.S. Further particulars and application forms from the Registrar, The University, Keele, Staffs. ST5 5BG, to whom completed applications should be sent by February 28, 1979.
9271(A)

LECTURER IN ORGANIC CHEMISTRY
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF SOUTHAMPTON

Applications are invited for the post of Lecturer in Organic Chemistry from October, 1979. Salary scale £3,883 to £7,754 per annum The initial salary will depend on qualifications and experience

Applications should include a curriculum vitae (seven copies), summary of research interests and names of two professional referees and should be sent as soon as possible and not later than February 28, 1979 to Mrs E. C. P. Sears, Staffing Section, The University, Highfield, Southampton SO9 5NH, from whom further particulars can be obtained. Please quote reference number 1606/R/Na
9272(A)

UNIVERSITY OF EXETER

A vacancy has arisen in the Department of Biological Sciences for a

TECHNICIAN
(Grade 3)

to work in the Electron Microscope Suite.

Applications are invited from persons with O.N C Sciences or equivalent qualifications and normally three years' experience

Salary £2,688 to £3,060 (under review)

Applications giving details of age, qualifications and experience, and naming two referees should be sent to Miss D. Watson, Northcote House, University of Exeter, EX4 4QJ by February 8, 1979 Please quote reference No. 5109.
9273(A)

BIOLOGY TEACHING OVERSEAS

Research-Teaching positions available beginning Fall 1979 in such specialties as Ecology, Plant Physiology, Developmental Biology, Invertebrate Zoology. Ph D required, rank and salary depend on experience and qualifications Usual contract is for three years with R T travel and other fringe benefits Curriculum vitae and letters of recommendation should be airmailed to Dr Samir Deeb, Biology Department, American University of Beirut, Beirut, Lebanon Application review will begin March 1, 1979
9276(A)

Computer Board For Universities And Research Councils

Joint Network Team
Appointment of Head

Recognising that flexible and compatible interconnection arrangements will play an important role in extending access to computing resources and expanding the range of facilities available to computer users in the academic community, the Computer Board and Research Councils are to set up a Joint Network Team. Its main aim will be to prepare, supervise and participate in a coordinated programme for the further development of computer networking among universities and Research Council institutes.

The specialist team is expected to consist of up to six posts and will be located at the Rutherford Laboratory, Chilton, Oxfordshire.

The responsibilities of the Head of the team will include presenting a programme of work to the Computer Board and the Research Councils for financial approval and supervising its execution. He will be called on to make recommendations to the sponsoring bodies on network plans for individual universities and Research Council institutes and to advise on technical and financial aspects of computer networking. An important part of the post will be to establish and maintain collaborative relationships with senior managerial and technical staff in computer centres throughout the academic community.

Applications are now invited from people with an interest in computer networking and with experience of university and Research Council computing. Applicants should have the ability to manage technical projects and a sound basic knowledge of computing and telecommunications technologies. Familiarity with current trends towards networking standards and proposed public packet-switched services would be an advantage.

The appointment will be for a fixed term of three years.

The salary will be in the range £10,746-£12,091 or £10,043-£11,300 depending on the superannuation scheme chosen.

Further details and an application form may be obtained from Jane Griffiths in the Personnel Group, Rutherford Laboratory. Please quote reference VN.760. Closing date for applications 12th February 1979.

Rutherford Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX.
Tel: Abingdon 21900.
9337(A)

SUNDERLAND POLYTECHNIC
FACULTY OF SCIENCE
DEPARTMENT OF BIOLOGY
LECTURER II IN MICROBIOLOGY

Applicants should preferably have a first degree in Microbiology and specialism in Microbial Physiology. Bacterial taxonomists or microbial ecologists with a strong physiological background may also be considered.

Salary Scale: Lecturer II £4,101 to £6,051 Bar to £6,558.

An application form and further details may be obtained from the Personnel Officer, Sunderland Polytechnic, Chester Road, Sunderland SR1 3SD, and should be returned as soon as possible.
9270(A)

PRINCETON UNIVERSITY

The Department of Biology has an opening at the level of

LECTURER

beginning in September 1979, to teach a laboratory course in experimental vertebrate biology which is complementary to an existing lecture course Areas of interest covered by the course include vertebrate reproductive biology with emphasis on neuroendocrine mechanisms Ample opportunity will be available to carry out individual research projects, preferably in the area of vertebrate reproductive biology, including neuroendocrinology. Ph D. degree or equivalent required.

Submit letter of application, curriculum vitae, and the names of three referees to Dr R D Lisk, Department of Biology, Princeton University, Princeton, N J 08540

Princeton University is an Equal Opportunity/Affirmative Action Employer.
9280(A)

UNIVERSITY OF ABERDEEN
DEPARTMENT OF FORESTRY
RESEARCH ASSISTANT

Applications are invited for the above post to work with Dr J A Petty on an investigation of the permeability to water of wood cell wall material in relation to both water transport in the living tree and industrial wood preservation Candidates should possess a good honours degree or a higher degree in biophysics, botany, chemistry, physics or wood science

The post is tenable for three years and is supported by the Leverhulme Trust Fund

Salary £3,384 to £4,882 per annum on Research and Analogous Staff Range 1B Scale, with appropriate placing

Further particulars from The Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by February 16, 1979
9264(A)

Australian Institute of Marine Science

Laboratory Manager

Applications are invited for the above position at the Australian Institute of Marine Science near Townsville in North Queensland. Facilities completed in 1977 include a 12 8 million dollar headquarters and comprehensively equipped laboratory complex, library, lecture theatre, controlled environment rooms and aquaria, accommodation for visiting scientists and various field and technical support facilities such as deepwater boat harbour, recompression chamber and maritime service building. The research fleet includes the 24.4m RV "Lady Basten" and various smaller craft

Research at AIMS pays comprehensive attention to an understanding of the physics, chemistry and biology of the marine environment with present emphasis on tropical systems

Duties:

To be responsible for the co-ordination of laboratory requirements, for instrument maintenance and liaison with research and workshop staff in laboratory services In particular to accept responsibility for the establishment and continuing management of an analytical services facility, extending to instrumentation employed in data collection at sea.

Qualifications:

A diploma or degree in chemistry with extensive experience in analytical instrumentation and procedures combined with skills in administration and personnel interactions relevant to the duties described. Experience relevant to physical and chemical oceanography would be an advantage

Salary:

A salary commensurate with the responsibilities of the position will be negotiated

Applications:

Applications must include full c v , plus the names and addresses of three professional referees and should be sent to:

The Secretary,
Australian Institute of Marine Science,
PMB No. 3,
Townsville M.S.O. Qld. 4810.

Closing date for applications is 28 February 1979. 9288(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(University of London)

Denmark Hill, London SE5 8RX

CHEMIST *or* BIOCHEMIST

required for exciting work on extraction and identification of lipids from mammalian tissues. Postdoctoral or equivalent experience essential Salary up to £5,579 per annum, two-year initially.

Please contact Professor Alan Bennett by telephone (01-274 6222, extension 2443) or write to Department of Surgery, King's College Hospital Medical School, Denmark Hill, London SE5 8RX 9305(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(University of London)

Denmark Hill, London SE5 8RX

DEPARTMENT OF DERMATOLOGY

RESEARCH ASSISTANT/ MEDICAL LABORATORY SCIENTIFIC OFFICER

required to work in the field of cytotoxic drugs to treat cancer and other skin diseases.

Some laboratory experience necessary Salary in the range £3,261 to £4,680 plus £354 London Weighting Allowance, according to age, qualifications and experience

For further information, please contact Dr A W du Vivier on 01-274 6222, extension 2521

Apply in writing, giving full curriculum vitae and the names and addresses of two referees to the Secretary of the Medical School at the above address. 9303(A)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF EXPERIMENTAL PSYCHOLOGY

Applications are invited for the office of

UNIVERSITY DEMONSTRATOR

IN THE DEPARTMENT OF EXPERIMENTAL PSYCHOLOGY

An interest in developmental aspects of Psychology will be an advantage but candidates with interests in other fields will be considered.

The initial appointment will be for three years from May 1, 1979, or as soon as possible thereafter The pensionable stipend for a University Demonstrator will be on a scale of £4,133 rising by four annual increments to £5,129, with initial placing above the minimum where appropriate The maximum tenure of a University Demonstratorship is five years A grant is made towards removal expenses

Candidates should send twelve copies of their application, together with the names of not more than three referees, to Mr G R Anderson, General Board Office, The Old Schools, Cambridge CB2 1TT, from whom further information can be obtained to arrive not later than March 15, 1979

9262(A)

Qualified Laboratory Scientific Officer required in Physiology Department, to assist with teaching laboratory duties and research. Salary not less than £3,261 on scale rising to £4,680, plus £354 London Weighting Apply in writing to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. P.Y.1. 9254(A)

ROYAL ALEXANDRA HOSPITAL FOR CHILDREN

Camperdown, N.S.W. Australia

BIOCHEMIST

Applications are invited for the new position of Biochemist (Principal Scientific Officer) within the Department of Biochemistry in the Hospital's Institute of Pathology.

The Hospital is a 400 bed Paediatric Teaching Hospital of the University of Sydney and offers a comprehensive range of clinical and laboratory services

The new appointee will assist the present Biochemist (Dr L Webster) in the routine work of the Department and will have special responsibilities in the development of new tests and techniques in the area of clinical paediatric chemistry. He/she will closely liaise with the Hospital's clinical staff and participate in the training programme of clinical and laboratory staff.

Applicants should hold a Ph D degree in Biochemistry or its equivalent; previous experience in clinical chemistry would be an advantage

Salary range $A21,049 to $A26,145 per annum

Further information is available from Dr R K Oates, Director of Medical Services

Applications including the names of two referees should be addressed to the undersigned as soon as possible

J. S. Yu, General Superintendent

9283(A)

Re-advertisement—

UNIVERSITY OF EXETER

Applications are invited for the post of

INSTRUMENT TECHNICIAN

(Grade 6)

in the Department of Biological Sciences, to provide a comprehensive service of maintenance, repair and calibration of a wide range of scientific instruments The work involves consultation with and advice to academic staff and postgraduates and instructing instrument users A chemistry background would be an advantage.

Salary £3,654 to £4,365 (under review)

Applicants should have an H N C qualification and appropriate experience in the work Further details can be obtained from the Laboratory Superintendent, Hatherly Laboratories, Prince of Wales Road, Exeter EX4 4PS It is not necessary for previous applicants to re-apply

Letters of application including the names and addresses of two referees should be sent to Miss Doreen Watson Northcote House, University of Exeter, by February 15, 1979 Please quote Reference No 5104 9274(A)

UNIVERSITY OF SASKATCHEWAN

DEPARTMENT OF PHYSIOLOGY

Applications are invited for an appointment as an Assistant Professor in the Department of Physiology Applicants must have an M D., D.V.M., Ph.D. or equivalent qualifications with at least two years of postdoctoral experience. Research experience in renal or gastrointestinal physiology is desirable. The appointee will be required to teach undergraduate and graduate level classes and to conduct an independent research programme

Salary will be determined by experience with a current floor to the range at $17,488 per annum The appointment commences on July 1, 1979. Detailed applications with curriculum vitae and naming three referees should be sent to Dr J W. Phillis, Head, Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, S7N 0W0, Canada 9260(A)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited for
post of

LECTURER

in Organic Chemistry

tenable from October 1, 1979,
January 1, 1980 The Department
well equipped for research and t
successful candidate will be expect
to undertake an active programme
teaching and research. The salary w
be according to age and experience
the scale £3,883 to £7,754 but it
likely that appointment will be ma
within the first three points of t
scale (£3,883, £4,133 and £4,332).

Applications, accompanied by t
names of three referees, should
returned before March 19, 1979,
the Staff Appointment Offic
Registrar's Department, University
Nottingham NG7 2RD, from wh
further particulars can be obtained.
9257(A)

ANIMAL DISEASES RESEARCH ASSOCIATION

MOREDUN INSTITUTE

SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER

There is a vacancy in the Patholo
Department for a person with expe
ence in cellular immunology to j
a team studying mucosal defe
mechanisms in sheep The appoin
would also help develop immunohis
chemical techniques for the identifi
tion of antigens on the cell surfa
and within tissues. Experience
serological and immunochemical tec
nique would be advantageous. App
cants should have an Ordinary Degr
H.N C or equivalent with experien
or Hons Degree in an appropri
subject. Five years' relevant po
qualifying experience is required
appointment as H.S O. Salary scal
S O £2,839 to £4,415, H.S O. £4,
to £5,448 with entry according to t
qualifications and experience of t
successful applicant. Non-contribut
superannuation scheme. Applicatio
giving the names of two refer
should be sent to the Secreta
Animal Diseases Research Associati
Moredun Institute, 408 Gilmert
Road, Edinburgh EH17 7JH, fr
whom further particulars may also
obtained. 9258(A)

THAMES POLYTECHNIC

SCHOOL OF MATERIALS SCIENCE AND PHYSICS

X-RAY and NUCLEAR LABORATORY TECHNICIA

(Grade 5)

Applications are invited for
Technician for the X-ray and nucl
laboratories who will be required
operate X-ray equipment, han
radioactive sources and be responsi
for keeping exposure records. Can
dates should have a recognised appr
ticeship or H.N D./H N C qualifi
tion and at least eight years' relev
experience

Salary scale £3,474 to £4,
inclusive.

Further particulars and form
application may be obtained from t
Staffing Officer, Thames Polytechn
Wellington Street, London SE18 6P
to whom completed applicatio
should be returned by January
1979. 9255(A)

M R C. Unit on the Developm
and Integration of Behaviour, Madi
ley, Cambridge CB3 8AA. A Resea
Assistant ½ to ¾ time is required
work on the development of so
behaviour. Some experience of
search in this area highly desira
Please apply in writing to Profe
R. A. Hinde at the above addres
9286(A

SCIENTIST—HORMONES

We require a postdoctoral scientist with a good honours degree in medicine, physiology, biochemistry or related discipline, to work in the Division concerned with endocrinology. The work involves research into the physiology and mechanisms of action of polypeptide hormones, development of methods to measure, characterise and control them, and setting up international (WHO) standards for their bioassay and immunoassay.

There are good opportunities for collaboration, both locally and internationally.

The appointment is for 5 years in the first instance. Salary is in the range £4,359 to £7,005 per annum on M.R.C. scales, depending on age and experience.

Please write or telephone for an application form quoting reference 0187 to the Personnel Officer, The National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB. Tel: 01-435 2232, ext. 211.

Applications should be returned not later than February 9, 1979.

9346(A)

UNIVERSITY OF THE WITWATERSRAND

Johannesburg

DEPARTMENT OF PHYSIOLOGY

Applications are invited from suitably qualified persons for appointment to the posts of:

(a) RESEARCH OFFICER/ JUNIOR RESEARCH OFFICER

Applicants for the post of Research Officer should have a medical or veterinary degree, or a Ph.D. in biochemistry, physiology, pharmacology, zoology, or any other biological field.

Applicants for the post of Junior Research Officer must have a B.Sc. or B.Sc. Honours degree in the above fields.

The successful applicants for both posts will be involved in an ongoing programme of research, at a biochemical, pharmacological, and physiological level, of cardiovascular physiology in health and disease. The Cardiovascular Research Unit is housed in a well-equipped modern cardiovascular laboratory, and current interests include work on the autonomic physiology and pharmacology of coronary artery disease and cerebrovascular disease, and the biochemical characterisation of adrenergic receptors.

(b) TECHNICIANS

Three technical posts in the Department of Physiology are vacant. One post is allocated for general Departmental duties, another for work in the University/M.R.C. Cardiovascular Research Unit, and the third in the Neurophysiology / Pain / Temperature Regulation Research Laboratory. Candidates should have a Diploma in Medical Technology, with some research laboratory experience, or should have at least a B.Sc. degree with a major in biochemistry, or any other field of biology.

The salary for the Research Officer for 1979 is up to R10,000 per annum and for the Junior Research Officer up to R7,500 per annum. Salaries for technical staff will depend on qualifications and experience.

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, 2001 South Africa, with whom applications should be lodged by February 16, 1979.

9304(A)

MEDICAL RESEARCH COUNCIL

LABORATORY ANIMALS CENTRE

NUTRITIONIST

Scientists with postdoctoral research experience in the nutrition of laboratory animals, farm livestock or poultry are invited to apply for the post of head of the Centre's Nutrition department. The successful applicant will be the focus for all aspects of the Centre's work in this field and will undertake research into the nutritional requirements of laboratory animals leading to general improvements in nutrition with particular emphasis on breding performance and the lowering of mortality rates. He or she will also provide a comprehensive advisory service to diet manufacturers and users of laboratory animals.

Excellent conditions of service, attracting a salary commensurate with age, qualifications and relevant experience.

Applications, enclosing a full curriculum vitae, should reach the Director, Laboratory Animals Centre, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, by March 1, 1979.

9344(A)

UNIVERSITY OF LONDON

INSTITUTE OF PSYCHIATRY

RESEARCH ASSISTANT

Applications are invited from recent graduates in biochemistry, pharmacology or a related discipline, to undertake a behavioural and biochemical investigation of the long-term effects of neuroleptic administration to rodents.

The position, which is supported by the Medical Research Council, is tenable for one year in the first instance, and may be renewed for up to three years.

Salary £3,384 to £3,883 per annum plus London Weighting.

Applicants may visit the Department (Department of Neurology) (Professor Marsden and Dr Jenner) by prior arrangement. Phone 01-703 5411, Extension 245.

Application forms available from The Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF. Phone 01-703 5411, Extension 228, quoting Ref: CM/N2.

9302(A)

MICROBIOLOGY FACULTY MEMBERS

The University of Kentucky College of Medicine has two faculty openings in the newly reorganised Department of Medical Microbiology and Immunology which are to be filled at the Assistant and Assistant or Associate Professor levels. Candidates should have a Ph.D. or M.D. and postdoctoral and/or faculty research experience. Senior-position candidates must have an ongoing funded research programme. Candidates are desired in the areas of microbial physiology, molecular genetics, molecular basis of microbial pathogenesis and gene regulation. Emphasis will be placed upon genetic, biochemical and molecular approaches to basic problems. Duties will include the development of a strong research programme, graduate student training and teaching of medical students. The positions will be available beginning approximately January 1980.

Send completed curriculum vitae including the names of at least three references to: S. Kaplan, Department of Medical Microbiology and Immunology, University of Kentucky College of Medicine, A. B. Chandler Medical Center, MN-524 Medical Center, Lexington, Kentucky 40536.

The University of Kentucky is an equal opportunity employer F/M.

9281(A)

Fibre Technology

G. D. Searle is a leading pharmaceutical company with its main U.K. site and research laboratories at High Wycombe in Buckinghamshire.

Within the laboratories we are involved in a growing number of long-term research and development programmes in various fields of medical science.

The following staff are required to develop new biocompatible materials for medical applications starting with a naturally occurring polysaccharide.

Polymer Chemist

Required to extract and chemically modify the naturally occurring polymers and to investigate alternative solvent systems. We are seeking a person with at least two years research experience working with polymers who possess a good honours degree and preferably a PH.D.

Fibre Scientist

Required to produce fibres using the wet and dry spinning techniques and to convert the fibres into knitted and woven products. Ideally we are looking for a person with proven research ability as applied to the commercial production of fibres and/or woven materials.

In addition to competitive salaries, conditions of employment include 4 weeks holiday per year, a contributory pension fund, subsidised private health insurance, sports and social club and staff cafeteria.

Applicants, male and female, should apply to Mr G. Jenkins, Personnel Manager, R & D Division, G. D. Searle & Co. Ltd., Lane End Road, High Wycombe, Bucks. (Tel. High Wycombe 21124 ext. 3374.) 9341(A)

SEARLE

POSTDOCTORAL RESEARCH ASSOCIATE

to participate in studies concerning processing of RNA and low molecular weight RNAs (gene organisation and regulation) in mammalian cells. These studies will involve cloning of DNA and sequencing, as well as other biochemical, physiological and genetic studies.

Position will be available from March 1, 1979.

Send curriculum vitae and names of three references to Dr David Apirion, Department of Microbiology and Immunology, Box 8093, Washington University School of Medicine, St Louis, Missouri 63110. Equal Opportunity and Affirmative Action Employer. M/F H.

9285(A)

TECHNICIAN/ RESEARCH OFFICER

required to assist in our Biological Safety Laboratory and the Cell Culture Laboratory. Microbiological training is essential. H.N.C. or Degree required. Salary range £3,600 to £5,200 inclusive London Weighting, pension scheme. For further information and application form, telephone Miss S. M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 on 242 0200, ext. 305.

9265(A)

UNIVERSITY OF SHEFFIELD

TECHNICIAN (GRADE 3)

required for the Department of Biochemistry. The person appointed will assist in research under Professor P. M. Harrison on the structure and function of the iron-storage protein ferritin. The work includes the isolation and analysis of protein ferritin from animal tissues by biochemical techniques and assistance in the X-ray crystallographic analysis of ferritin structure (molecular model building, drawing of maps, computation, photography). Assistance in the preparation of some laboratory classes is also expected.

Minimum qualifications: O.N.C. in Applied Biology and/or Chemistry.

Salary on scale £2,688 to £3,060 per annum (under review).

Please write to the Administrative Officer (Personnel) (Ref. S1145/N). The University, Sheffield S10 2TN.

9320(A)

GRADUATE RESEARCH ASSISTANT required for work in Tissue Culture and Cytogenetics Laboratory. Experience in cell culture, cytogenetics or microbiology would be an advantage. Applications to be sent to the Administrative Assistant, Paediatric Research Unit, The Prince Philip Research Laboratories, Guy's Tower, London Bridge SE1 9RT.

9295(A)

CSIRO AUSTRALIA

Research Scientist

Division of Fisheries & Oceanography Perth, WA

CSIRO has a broad charter for research into primary and secondary industry areas The Organization has approximately 7000 employees—2400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia

Field: Biological Oceanography

General: The Division is Australia's principal marine research institution with about 70 scientists investigating the physical, chemical and biological features including fisheries of the oceans around Australia Headquarters of the Division are at Cronulla, Sydney, with regional laboratories in Perth, Brisbane and a field laboratory at Karumba On-line access to a Cyber 76 computer is available. Two well-equipped medium sized chartered vessels are available for oceanographical research and there are several smaller CSIRO vessels.

Duties: To initiate and carry out research in primary and secondary productivity of limestone reefs off S W Australia, with particular attention to the interaction between offshore and onshore influences as determinants of the stability of the benthic ecosystem

Qualifications: A marine ecologist is required with experience in plant and animal physiological and biochemical ecology, and knowledge of biochemical techniques, and the use of radioactive isotopes Minimum formal qualifications are a PhD or equivalent.

Salary: Research Scientist: $A14829—$A18177 p a or Senior Research Scientist $A18819—$A21543 p.a.

Tenure: Indefinite with Superannuation

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 320/608 should reach—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 24th February 1979.

Applications in U S.A and Canada should be sent to—The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W , Washington, D.C , 20036, U S.A. 9289(A)

INSTITUTE OF GEOLOGICAL SCIENCES

GEOCHRONOLOGIST

The Institute has a vacancy based in London for a geochronologist to undertake geological age determination studies in the U K and overseas, and to further the development of geochronology and isotope geochemistry by research into analytical methods and the interpretation of analytical data It is anticipated that work on material from overseas will constitute a major part of the activities of the post and will involve short periods of field work in developing countries Work overseas will involve co-operation with Geological Surveys and with I.G S manned technical assistance project.

QUALIFICATIONS

Applicants should normally be under 32 and have a good honours degree in geology or the physical sciences with post graduate experience in at least two methods of geological age determination work. Grade of appointment and starting salary will depend on qualifications and experience.

SALARY

Scientific Officer	£2,839 to £4,415
Higher Scientific Officer	£4,101 to £5,448
Senior Scientific Officer .	£5,154 to £6,898

Starting salary may be above the minimum. The post attracts Inner London Weighting Allowance of £465 per annum Non-contributory pension scheme.

The staff of Council are not Civil Servants but their pay and conditions of service are similar to those of scientists in the Civil Service.

For an application form to be returned by February 23, 1979, write to Establishments (Recruitment) Section, Institute of Geological Sciences, Exhibition Road, London SW7 2DE.

Please quote reference SO/IG/79/1.

NATURAL ENVIRONMENT RESEARCH COUNCIL 9306(A)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following

JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

DEPARTMENT OF CLINICAL SCIENCE

RESEARCH FELLOWS

(2 positions)

Candidates for these positions should be graduates in medicine or science with appropriate postgraduate training The Department is currently undertaking biochemical and pharmacological studies on membrane function in relation to human disease Emphasis is on calcium, cyclic nucleotide and prostaglandin metabolism, asthma, diseases of muscle, malignant hyperpyrexia, platelet function, atherosclerosis, thrombosis and haemostasis, osteogenesis imperfecta, clinical pharmacology and clinical immunology

Appointment will normally be for two years but in exceptional circumstances may be for three years and extended to a total period of five years. It is hoped that the successful candidates will be able to take up their appointments in the first half of 1979.

Applicants should arrange for reports from three academic referees to be sent to the Registrar of the University in support of their applications

A salary loading of up to $A5,000 per annum may be paid to an applicant holding appropriate medical qualifications.

Further information may be obtained from the Acting Head of the Department, Dr M. A Denborough, in the University

CLOSING DATE· **February 28, 1979.**

DEPARTMENT OF IMMUNOLOGY

SENIOR RESEARCH FELLOWS/RESEARCH FELLOWS

Two positions are available for suitably qualified scientists The Department is doing research into the genetic basis of disease resistance in livestock, the major histocompatibility complex of cattle, the immunological aspect of reproduction, foetal-maternal interactions, the physiology of the immune response, transplantation biology, the immunobiology of cancer, and immunological tolerance Scientists doing research into any of these areas either from the physiological, biochemical or cellular standpoints will be considered

A salary loading of up to $A5,000 per annum may be paid to an applicant holding appropriate medical qualifications.

CLOSING DATE **February 28, 1979.**

SALARIES· Salary on appointment will be in accordance with qualifications and experience within the ranges Senior Research Fellow $A22,049 to $26,301, Research Fellow $A15,786 to $20,606 per annum Current exchange rates $A1=58p=$US1 14

Tenure: Appointment as Senior Research Fellow is normally for 3 years in the first instance with the possibility of extension to 5 years unless otherwise stated.

Other conditions: Reasonable appointment expenses are paid

Superannuation benefits are available for applicants who are eligible to contribute Assistance with finding accommodation is provided for an appointee from outside Canberra

The University reserves the right not to make an appointment or to make an appointment by invitation at any time

Prospective applicants should obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 9313(A)

UNIVERSITY OF BATH

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for an

EXPERIMENTAL OFFICER

supported by the Science Research Council to work on the structure and immunology of mammalian cell membranes with particular reference to the glycoprotein components The milk fat globule membrane is being used as a convenient large scale source of mammalian membrane and its immunochemical characterisation is currently underway.

The tenure of the post is one year Experience in gas chromatographic analysis of carbohydrates and/or immunochemistry would be an advantage, but is not essential.

Salary range £2,688 to £3,402 per annum (under review)

Application forms and further particulars from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting reference number 79/10/N Closing date will be February 2, 1979 9259(A)

PRINCETON UNIVERSITY

The Department of Biology has an opening at the level of

LECTURER

beginning in September 1979, to supervise a laboratory course in introductory biology Opportunity is available to carry out independent research, depending on the qualifications of the candidate. Submit letter of application, curriculum vitae, and the names of three referees to Prof Edward C. Cox, Department of Biology, Princeton University, Princeton, N J 08540

Princeton University is an Equal Opportunity/Affirmative Action Employer 9308(A)

CENTRAL PUBLIC HEALTH LABORATORY

National Collection of Type Cultures (N C.T.C.)

SYSTEMS DESIGNER/PROGRAMMER

A systems designer/programmer, preferably a science graduate, is required for the operation of a bacterial identification service. Bacteria from clinical sources are identified by probabilistic analysis of test results by reference to computer-stored data bases Systems use Optical Mark Reader forms, Data Recognition (Dataterm 3) Machine, Extel punch tape terminal connected to the LUCS 6500 time sharing system current software in FORTRAN IV There is considerable scope for improving the responsiveness of the system and the efficient storage and retrieval of data, and to revise the methodology or explore alternative methods Sympathetic consideration would be given for specialised training, if required

Grading dependant upon basic qualifications in appropriate subjects and on experience

Salary scales. Scientific Probationary scale £2,991 to £4,275 plus London Weighting (£354); Post Probationary scale £3,798 to £4,899 plus London Weighting or Senior Scientific scale, if applicable, £5,451 to £6,837 plus London Weighting N H.S. Designated scale 9 £4,959 to £6,025 plus London Weighting

Ring 01-205 7041 for a more detailed job description (Dr L. R. Hill, on Extn. 47) and for an application form (Personnel Officer, on Extn. 12)—Central Public Health Laboratory, Collindale Avenue, Collindale, London, N.W.9 Closing date: February 15, 1979. 9293(A)

THE UNIVERSITY OF MANITOBA

invites applications and nominations for the position of

DEAN OF THE FACULTY OF SCIENCE

The Faculty of Science at The University of Manitoba has a complement of 180 faculty and 90 support staff. Its twelve departments and units span the Biological, Mathematical and Physical Sciences; they offer a full range of undergraduate Science programmes for approximately 2,100 students enrolled in the Faculty, courses for a large number of students from other faculties and schools and M.Sc. and Ph.D. programmes for about 280 graduate students.

Candidates should have achieved distinction as scholars in a major field of Science, have a commitment to advancing higher education in Science and have strong leadership ability. Relevant administrative experience and knowledge of the Canadian educational system would be assets.

The appointment is expected to commence September 1, 1979, and will be for a renewable term of normally five to seven years.

Please address responses to:

Provost R. A. Johnson,
Chairman,
Selection Committee for Dean of the Faculty of Science,
Room 202, Administration Building,
The University of Manitoba,
Winnipeg, Manitoba,
Canada, R3T 2N2.

Applications, nominations and suggestions will be received until March 15, 1979. 9171(A)

ROYAL DENTAL HOSPITAL OF LONDON SCHOOL OF DENTAL SURGERY

(University of London)

32 Leicester Square, London WC2H 7LJ

Applications are invited for the post of Lecturer in Dental Materials Science in the Department of Physical Sciences of the School The appointment will be to the permanent academic staff of the School and the successful candidate will participate fully in all aspects of the work of the Department, including undergraduate and postgraduate teaching and research, for which adequate facilities are available Applicants should have good academic qualifications and research experience in materials science or a related field.

Salary will be within the range £3,906-£7,764, plus London Allowance of £450 per annum, superannuable The successful candidate will be expected to take up duty as soon as possible Applications with full curriculum vitae and names of three referees should be submitted to the School Secretary not later than 16 FEBRUARY 1979. 9314(A)

POSTDOCTORAL POSITIONS PREDOCTORAL POSITIONS

The University of Kentucky College of Medicine has both postdoctoral and predoctoral positions available within the Department of Medical Microbiology and Immunology for individuals interested in the genetic control of membrane development and biosynthesis. Postdoctoral applicants should send a completed curriculum vitae including three letters of reference. Postdoctoral salary will be $11,000-$12,000 per annum depending upon experience. Predoctoral applicants should write for information Both teaching and research assistantships are available.

All inquiries should be sent to S Kaplan, Department of Medical Microbiology and Immunology, University of Kentucky College of Medicine, A. B. Chandler Medical Center, MN-524 Medical Center, Lexington, Kentucky 40536.

The University of Kentucky is an equal opportunity employer F/M 9282(A)

GUY'S HOSPITAL MEDICAL SCHOOL

(University of London)

POSTDOCTORAL RESEARCH ASSISTANT

required to join a research group under Dr L. Mallucci in the Department of Microbiology investigating the role of cell surface membrane components in cell growth regulation and cell differentiation Position available to December 1979 with the possibility of further renewal of grant.

Salary in range £3,910 to £5,506 plus £450 London Allowance and superannuation.

For further details telephone Dr Mallucci

Applications to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT. 9055(A)

Synthetic Organic Chemists

Medicinal and Animal Health Research

We require two additional Ph.D. organic chemists with several years' experience at the post-doctoral level, who are able to demonstrate a sound record of creativity and who possess those personal qualities necessary to stimulate and catalyse good ideas with other members of the project team with whom they work.

Our projects are organised on multi-disciplinary bases and it is most important therefore that the successful candidates can think well beyond the purely chemical field and be able to originate synthetic programmes based on biological concepts. Effective communication with colleagues in other disciplines is vital. We would expect those appointed to be capable of organising and running a project of their own within the forseeable future.

The appointments carry starting salaries in the £6/8000 range and in addition to offering excellent career prospects there is good bonus potential. Pension, death benefit and salary continuance schemes are in operation, and there is generous assistance with relocation expenses to a pleasant coastal/rural area. Flexible hours are worked and the social/recreational facilities, which include squash, sailing, etc. are first class.

Pfizer

Applications from men and women, which will be treated in confidence, giving brief details of qualifications, experience, age, etc. should be sent to:

**D. W. Sells, Pfizer Central Research,
Sandwich, Kent, CT13 9NJ.**

9277(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

FACULTY OF MEDICINE

ASSISTANT PROFESSOR OF IMMUNOLOGY

Immunologist/Immunogeneticist required at Assistant Professor (and Postdoctoral fellow) level to join others in a medical school immunology group Special research strengths of this group are a good HLA lab (International workshop standard), complement allotyping, collaborative relationships with the blood bank, biochemical genetics, cytogenetics and with clinical investigators, plus the availability of large families Our group is also involved in teaching, in a clinical immunology diagnostic laboratory and with a transplantation programme

For more information please write, preferably sending detailed curriculum vitae plus any relevant reprints, to Dr W H. Marshall, Professor of Immunology, Faculty of Medicine, Memorial University of Newfoundland, Canada, A1B 3V6. 9137(A)

UNIVERSITY OF CAMBRIDGE

PROFESSORSHIP OF MATHEMATICAL PHYSICS (1967)

Applications invited for above Chair which will become vacant on October 1, 1979, by the resignation of Professor J C Polkinghorne. The Professorship is primarily assigned to the Faculty of Mathematics and the Professor will be accommodated in the Department of Applied Mathematics and Theoretical Physics Preference will be given to candidates whose work is conected with theoretical elementary particle physics.

Present pensionable stipend £10,939.

Applications (10 copies) marked 'Confidential' should be sent to the Secretary General of the Faculties, from whom further information may be obtained, at the General Board Office, The Old Schools, Cambridge CB2 1TT. Names of two referees may be submitted if desired.

Closing date: February 28, 1979. 9345(A)

We wish to appoint someone with experience in the medical or biological sciences to head a fully established research team in our Preclinical Research Department. The work of this team is mainly concerned with gerontological and pharmacological problems.

The person appointed will have a university degree in the medical or biological sciences backed by practical experience in the fields of anatomy, pathology or brain research. Importance is also attached to a knowledge of morphometry and/or stereology and to a good command of the English language.

Applicants are asked to send a short letter of application in the first instance to:

SANDOZ

Sandoz Ltd., Personnel Dept. Ref. 1200,
Postbox, 4002 Basle, Switzerland. 9321(A)

Cambridgeshire Area Health Authority (Teaching)

**Addenbrookes Hospital
Cambridge**

Applications are invited for the NHS post of

Senior Biochemist

in the Department of Clinical Biochemistry, which is comprised of both NHS and University elements. This post is primarily concerned with diagnostic services. The laboratory acts as a regional centre for a number of analytes and has excellent facilities for research. Candidates should possess a higher qualification and at least 4 years of relevant experience. Further training for final M.C.B. and M.R.C. Path. qualifications is available. Whitley Council conditions of service; salary scale £5,451 - £6,837.

Enquiries relating to the post and requests for application forms should be addressed to Professor C. N. Hale, Department of Clinical Biochemistry, Addenbrookes Hospital, Hills Road, Cambridgeshire, CB2 2QQ. Tel: 0223 45151. Ext. 7152.

The closing date for applications is March 1. 9294(A)

UNIVERSITY OF EXETER

CHAIR OF PHILOSOPHY

Applications are invited for the Chair of Philosophy, made vacant by the retirement, on September 30, 1979, of Professor D. J. O'Connor. No field of specialisation is prescribed for the Chair. Salary will be within the agreed professorial range, currently £9,443 minimum, £11,055 average, per annum (under review).

Further particulars should be obtained from the Academic Registrar and Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter EX4 4QJ. Closing date for receipt of applications (13 copies, overseas candidates 1 copy), February 26, 1979. Please quote reference number 3197. 9309(A)

Ph. D. DRUG METABOLISM

In recent years the discovery and development of new drugs has lead to an increasing demand for insight regarding their safety and mode of action. It has been recognised that the species differences in drug responses and toxicity which complicate the evaluation of drugs in laboratory animals can often be explained by corresponding species differences in the metabolism of the drug. Thus, the investigation of the absorption, distribution, metabolism and excretion of drugs in laboratory animals and man leads to a better understanding of the mode of action and toxicology. This briefly is the work of our Drug Metabolism Department.

We are now looking for an additional Ph.D with a broad understanding of pharmacology and drug research who has a strong desire to use this knowledge in drug discovery, and who wants to interact closely with multidisciplinary discovery teams.

The appointment, which is particularly suitable for a man or woman with 2/3 years post-doctoral experience, offers excellent career prospects, a competitive salary and good bonus potential. Pension, death benefit and salary continuance schemes are in operation and there is generous assistance with relocation expenses. Flexible hours are worked and the social/recreational facilities, etc., are first class.

Pfizer

Applications, which will be treated in confidence giving brief details of qualifications, experience, age etc., should be sent to:-

**D. W. Sells,
Pfizer Central Research,
Ramsgate Road,
Sandwich,
Kent CT13 9NJ** 9348(A)

Senior Research Position N/400

Basic research, preferentially in molecular genetics of eukaryotic systems, molecular immunology, cell biology or neurosciences. The position will be equipped with sufficient investment funds and with the possibility to start a group consisting of one technician, one postdoctoral coworker and students. University affiliation possible. Cooperation with existing groups in DNA repair, cell biology and mutagenicity desirable.

Applications should include a personal record, a list of publications and a brief description of intended research.

Postdoctoral position N/400

Biochemical and molecular characterization of immune deficiencies, within new research group: H. Ponta, H. J. Rahmsdorf and P. Herrlich. Applications should include a personal and scientific record including list of publications.

Deadline: March 1, 1979

Address: Kernforschungszentrum, IGT, Postfach 3640, D 7500 Karlsruhe, Germany.

**Kernforschungszentrum
Karlsruhe GmbH**

9340(A)

Experimental Scientists

Neutron Scattering Instrumentation.

Rutherford Laboratory.

A new intense source of pulsed neutron beams, the spallation neutron source is being built at the Rutherford Laboratory for use in research programmes of U.K. universities and polytechnics. A substantial number of neutron scattering instruments will be provided based on the time-of-flight method. A wide range of equipment and techniques is involved; for example, position sensitive detectors for epithermal neutrons; high resolution choppers; sample environments controlling parameters such as temperature, pressure, magnetic field; neutron guides; neutron polarisation systems.

There are vacancies in the Neutron Beam Research Unit for experimental scientists to participate in the development and provision of this equipment. As part of their development activities those appointed will have opportunities to work with research scientists using instruments at Harwell and the Institut Laue-Langevin, Grenoble. Applicants should have a degree in physics or applied physics, or an equivalent qualification, and preferably some experience in fields such as electronics, high vacuum, cryogenics. Appointments will be made to permanent positions in the Science Group at the level of Scientific Officer or Higher Scientific Officer.

Salary Scales: Scientific Officer £3,037-£4,724
Higher Scientific Officer £4,388-£5,829

Please apply to Jane Griffiths in the Personnel Group on Abingdon (0235) 21900 Ext. 510 or write to her quoting Reference No. VN 768.
Closing date for applications –16th February, 1979.

RUTHERFORD
Science Research Council
Rutherford Laboratory, Chilton, Didcot, Oxfordshire OX11 OQX. Tel Abingdon 21900

9349(A)

Botswana

Forest Officer

Up to £10,100 Plus Allowances

A graduate in forestry, preferably with 5 years relevant post-qualification experience, is required for the management of indigenous forests and the protection of forest resources in this developing African country.

Salary includes a substantial tax-free allowance paid under Britain's overseas aid programme. Basic salary attracts a 25% tax-free gratuity.

Benefits include free passages, generous paid leave, children's holiday visit passages and education allowances, subsidised housing, appointment grant and interest-free car loan.

The terms on which civil and public servants may be released if selected for appointment will be subject to agreement with their present employers.

For full details and application form write quoting MD/149/NA.

Crown Agents

The Crown Agents for Oversea Governments and Administrations, Recruitment Division,
4 Millbank, London SW1P 3JD

9350(A)

IMMUNOLOGIST/ VIROLOGIST

Two positions are available immediately at the Assistant Professor level for a Cellular Immunologist and a Virologist to work in the Bovine Leukemia Research Unit. Applicants should have a Ph.D. or D.V.M. degree with post-graduate training in the appropriate discipline.

Qualified applicants should send a résumé, copies of representative publications and the names of three people who may be contacted for references to: Dr Jorge F Ferrer, Chief, Section of Viral Oncology, University of Pennsylvania School of Veterinary Medicine, New Bolton Center, R.D 1, Kennett Square, Pa. 19348 9298(A)

FELLOWSHIPS

A RESEARCH FELLOWSHIP

tenable for a period of up to three years, is available for work on the regulation of eukaryotic genes by steroid hormones. Emphasis will be given on the isolation, cloning and characterisation of specific genes. Candidate should be experienced in the techniques of nucleic acid biochemistry. Applications (with curriculum vitae) should be sent to Dr J.-P. Jost, Friedrich Miescher-Institut, P O Box 273, CH-4002 Basel, Switzerland

9227(E)

UNIVERSITY OF EXETER

DEPARTMENT OF PHYSICS

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from suitably qualified persons for an S.R.C. Postdoctoral Research Fellowship. The successful candidate will conduct research in Low Temperature Physics working with Professor A. F. G. Wyatt and Dr D. R. Allum. The experiments involve propagating high frequency phonons through liquid helium cooled by a dilution refrigerator. Present studies include a precise phonon group velocity measurement, angular phonon acceptance at a single crystal interface, non linear effects in the helium and at interfaces and atomic desorption by phonons.

The grant has been awarded for three years and the post is to commence as soon as possible.

Commencing salary will be in the range £3,883 to £4,382 per annum. The post is superannuated.

Further particulars may be obtained from Professor A. F. G. Wyatt, Department of Physics, University of Exeter, Exeter EX4 4QL, to whom applications, two copies (one copy from candidates living overseas) and the names of three referees should be sent by February 21, 1979 9325(E)

UNIVERSITY OF WARWICK

RESEARCH FELLOWSHIP IN VIROLOGY

Applications are invited for a Postdoctoral Fellowship in the Tumour Virus Research Group in the Department of Biological Sciences. The successful applicant will work in a small group studying the biochemistry of C type RNA tumour viruses, with particular reference to their ability to transform cells. Experience of nucleic acid technology and in particular the use of *in situ* hybridisation techniques, while not essential, would be an advantage. Starting salary will be between points 1 and 6 on the Research 1A scale £3,883 to £6,555 per annum. The Fellowship (funded by the Medical Research Council) is available from March 1, 1979 and is renewable on an annual basis until September 1981. Further details and application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref No. 24/2A/79. Closing date for receipt of applications is February 15, 1979. 9269(E)

UNIVERSITY OF CAMBRIDGE

Broodbank Fellowships

Applications invited for Fellowships for full time research normally at Cambridge in Biochemistry or Biophysics with special reference to the principles and practice of food preservation. Tenure normally from October 1, 1979, for one year first instance but may be extended for one or two further years. Candidature open, but preference for post-doctoral applicants. Pensionable salary for postdoctoral Fellow in range £3,425 to £5,058 according to experience and qualifications. Compulsory superannuation.

Applicants should ascertain that there is a laboratory in Cambridge willing to accommodate them and must outline research proposed indicating bearing on principles and practice of food preservation and include full curriculum vitae, copies of papers published or references thereto and names and addresses of at least two referees.

Full details available from and applications (ten copies) to reach the Secretary to the Managers of the Broodbank Fund, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX not later than March 1, 1979. 9261(E)

FELLOWSHIPS—continued

Commonwealth of Australia

QUEEN ELIZABETH II FELLOWSHIPS

Physical & Biological Sciences

To commemorate the Royal Visit to Australia in 1963 the Australian Government established the Queen Elizabeth II Fellowships scheme. Under this scheme up to ten fellowships may be awarded each year for full-time research by young scientists of exceptional promise and proven capacity for original work. These are post-doctorate awards tenable in an Australian university or approved research institution normally for two years. Tenure of a Fellowship is expected to commence within nine months of the announcement of the award.

QUALIFICATIONS: Queen Elizabeth II Fellows must be either Australian or United Kingdom citizens. They should have a Ph.D., or equivalent qualifications, in one of the physical or biological sciences (which are deemed to include mathematics and the scientific aspects of statistics, engineering, metallurgy, agriculture and medicine). Awards will, in general be restricted to applicants who are not more than 30 years of age on the date when applications close. Applications will also be considered from candidates for the Ph.D. degree if their thesis has been submitted for examination.

STIPEND: $17 131 (Australian) per annum – increased to $18 403 per annum at age of 28 years.

ALLOWANCES: Are payable in respect of a Fellow's dependent spouse ($500p.a.) each dependent child ($200), superannuation payments (up to 10 per cent of stipend), appropriate insurance coverage and necessary travel expenses.

APPLICATIONS: *Persons interested in applying for the above fellowships should obtain application forms and a statement of the conditions of award from the Secretary, Queen Elizabeth Fellowships Committee, Department of Science, P.O. Box 449, Woden A.C.T. 2606, Australia: the Minister (Scientific), Canberra House, 10–16 Maltravers Street, London, WC2R 3EH, U.K.; or the Australian Embassy, 1601 Massachusetts Avenue, Washington D.C. 20036, U.S.A. Applications for the next round of awards, which will be announced in June 1979 close at the Canberra address on 16 March 1979.*

9323(E)

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY

SPRING 1979 AWARDS

Next deadline: February 20, 1979

The long term fellowships will usually be for a period of up to one year but applications for a renewal for a second year are considered. To be eligible a candidate must hold a doctor's degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel. EMBO long term fellowships are **not**, however, awarded for exchanges between laboratories within any one country. Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel.

The deadline for applications for the 60 fellowships to be awarded in April 1979 is February 20, 1979. Because the selection procedure involves an interview this deadline should be respected.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R. Germany. 9278(E)

THE ROYAL SOCIETY

LOCKE RESEARCH FELLOWSHIP
ROSENHEIM RESEARCH FELLOWSHIP

Applications are invited by the Council of the Royal Society for:

(a) A Locke Research Fellowship for research in experimental physiology and pharmacology.

(b) The Rosenheim Research Fellowship established under the will of the late Mrs M. C. Rosenheim for research on the biochemistry of plants and the simpler forms of animal life. Preference will be given to candidates wishing to study the biochemistry of higher plants.

Both appointments are tenable in any appropriate university department or research institution approved by the Council of the Royal Society (the Rosenheim Research Fellowship is restricted to places in Great Britain). Candidates should supply the usual personal details and two referees. Testimonials will not be considered. The subject of the proposed research and where it will be done, together with the name of the head of the department, whose consent should first be obtained, must be stated.

The appointments, which will be subject to the Society's general regulations governing research appointments, will be tenable for three years in the first instance from October 1, 1979, and may be renewable for a further two years, such renewal being considered at the end of the second year of tenure. Applicants should preferably be under 33 for the Locke Research Fellowship and under 26 for the Rosenheim Research Fellowship on October 1, 1979. Stipend scales are as follows (the point of entry will be determined by Council), and superannuation benefits are provided:

Locke Research Fellowship (Category C): £6,153 by £240 to £7,113 by £243 to £7,356 per annum.

Rosenheim Research Fellowship (Category D): £4,461 by £249 to £5,706 per annum.

Applications should be made on forms to be obtained (stating name of Fellowship) from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and should be received not later than February 28, 1979. 9275(E)

FELLOWSHIPS—continued

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY

SPRING 1979 AWARDS

Next deadline: February 20, 1979

The long term fellowships will usually be for a period of up to one year but applications for a renewal for a second year are considered To be eligible a candidate must hold a doctors degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel, EMBO long term fellowships are not, however, awarded for exchanges between laboratories within any one country. Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel.

The deadline for applications for the 60 fellowships to be awarded in April, 1979, is February 20, 1979. Because the selection procedure involves an interview this deadline should be respected.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R. Germany. 9312(E)

POSTDOCTORAL RESEARCH ASSOCIATE

required to work on DNA-protein interactions, on the structure of chromatin in Dr J. G. Brahms' research group, in Paris. The group has a computerised Raman Laser spectrometer, vacuum U. V C. D. and other equipment and is actively engaged in structural and functional investigation on DNA and protein interaction in chromatin Some research experience in biochemical and biophysical techniques is desirable. Fellowship starting £5,000 per annum.

Application indicating training and research experience should be sent to Dr J. G. Brahms, Institut de Recherche en Biologie Moléculaire (Tour 43) 2, place Jussieu—75005 PARIS—France. 9266(E)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF CHEMISTRY

Applications are invited for a

POSTDOCTORAL RESEARCH FELLOWSHIP

in the Department of Chemistry, Southampton University. The person appointed will work with Dr B. Cleaver on a Science Research Council supported project to study the influence of high pressure on miscibility in high temperature liquid systems (molten salts and molten metals), at pressures up to 25 kbar and temperatures to 1,200 °C. The experimental work will be carried out mainly at Southampton, but will also involve the use of a new high pressure facility at the National Physical Laboratory, Teddington, for a six month period.

Applicants should hold a Ph D degree and should have experience in at least one of the following fields: molten salt chemistry; high pressure technology; thermodynamics of liquid systems. A driving licence would also be an advantage.

The post is available for two years, starting immediately. The starting salary will be in the range £3,883 to £4,382 (under review), depending on age and experience. Applications with curriculum vitae and names of two referees, should be sent to Dr B. Cleaver, Department of Chemistry, University of Southampton, from whom further particulars can be obtained. Please quote reference number 1608/R/Na. 9310(E)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF PHYSICS

RESEARCH FELLOWSHIPS IN CRYOGENICS

Applications are invited for postdoctoral fellowships to work on the following projects:

(1) Heat transfer in the reboiler/condensers of cryogenic plant, using model rigs and a full length test section.

(2) Complex convective flow in stratified cryogenic vapours and liquids using Laser Doppler Anemometry.

(3) Heat transfer and fluid behaviour of helium at 3,000 r.p.m. for the development of large superconducting machines.

Applications should have experience in mechanical/chemical engineering or low temperature physics

Salary in range up to £5,367 plus U.S.S. benefits.

Applications with names of two referees should be sent to Mrs E. C P. Sears, Staffing Section, The University, Highfield, Southampton SO9 5NH. Please quote reference no. 1607/R/Na. 9290(E)

STUDENTSHIPS

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research

POSTGRADUATE STUDENTSHIPS

The National Institute for Medical Research has positions for postgraduate students to study towards the degree of Ph.D. The fields of interest are broadly those covered by the following divisions and laboratories of the Institute, namely: Bacterial Infectivity, Biochemistry, Biophysics, Developmental Biology, Endocrinology, Genetics, Immunology, Leprosy and Mycobacterial Research, Microbiology, Molecular Pharmacology, Neurobiology, Neurophysiology and Neuropharmacology, Peptide Chemistry, and Virology.

The Institute invites applications from students in their final year in all branches of the physical and biological sciences. In general, approximately ten positions are available each year

For further information and application form please write stating your research interests to: Dr J. B. Ward, Director of Studies, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA. 9333(F)

BRITISH MUSEUM (Natural History)

POSTGRADUATE RESEARCH STUDENTSHIPS 1979

Three studentships will be available, tenable for 3 years, at the British Museum (Natural History) and commencing October 1979.

Arrangements will be made with an appropriate university for higher degree registration and joint supervision of the research project.

The research projects for which applications are invited are as follows:—

DEPARTMENT OF BOTANY

1. Fine structure and cytochemistry of reproduction in the seagrass *Zostera*.

DEPARTMENT OF ENTOMOLOGY

2. Phylogenesis of *Danaus* butterflies, as demonstrated by numerical taxonomy, hybridisation and Hennigian phylogenetic systematics.
3. Comparative anatomy of the reproductive system in aphids.

DEPARTMENT OF ZOOLOGY

4. Taxonomy and zoogeography of polymorphic cyclopoid copepods.

Facilities are available for scanning and transmission electron microscopy serology, biochemistry and biometrics In addition, students will have access to the Museum's extensive library.

ELIGIBILITY

Applicants to be aged 27 or less, ordinarily resident in Great Britain, and to have a first or upper-second class honours degree; students graduating in 1979 can be offered awards subject to their degree results reaching this level.

Students interested in applying should write to the Secretary (Postgraduate Studentships), British Museum (Natural History), Cromwell Road, London SW7 5BD, for application forms, conditions of the awards, and for further information on projects.

Closing date for receipt of completed applications forms—March 18, 1979.

8249(F)

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research

LABORATORY OF PEPTIDE CHEMISTRY

Applications are invited for a Science Research Council

C.A.S.E. STUDENTSHIP

to work with Dr D. G. Smyth on the synthesis and metabolism of novel analogues of peptides which occur in brain and pituitary. The project is sponsored by the Pharmaceutical Division of Reckitt and Colman and is tenable from October 1, 1979.

Applicants should be chemists or biochemists and should have, or expect to obtain, a good honours degree or equivalent. The successful applicant will be expected to enrol for a Ph.D. degree at the University of London

For further details please write as soon as possible to the Director of Studies, National Institute for Medical Research, Mill Hill, London NW7 1AA, enclosing curriculum vitae and the names of two referees 9332(F)

UNIVERSITY OF ESSEX

DEPARTMENT OF CHEMISTRY

Applications are invited for an

S.R.C. C.A.S.E. RESEARCH STUDENTSHIP

tenable for three years on the "Initiation of Liquid Hydrocarbon/Air Mixtures": in collaboration with Shell Research Limited, Thornton Research Centre, Chester.

Projects will be supervised by Professor J N. Bradley, from whom further details are available. Applications should be sent to Professor Bradley, Department of Chemistry, University of Essex, Wivenhoe Park, Colchester, as soon as possible.

9296(F)

University of Wales

UNIVERSITY COLLEGE OF SWANSEA

C.A.S.E. STUDENTSHIP

Applications are invited from persons holding, or expecting to obtain, a good honours degree in chemistry for a C.A.S E. Studentship in organic chemistry research towards the degree of Ph.D. on the project, **Oxidative Methods of Aromatic Substitution,** in collaboration with I.C.I (Organics Division). The project involves the synthesis of heterocyclic organic compounds by oxidative methods —e.g. electrochemically or by metal ions. The compounds will be of particular interest to the dyestuffs industry.

Further details are available from Dr T W. Bentley, Department of Chemistry, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom applications should also be sent along with curriculum vitae and the names of two referees. 9338((F)

SYMPOSIA

STRAW DECAY SYMPOSIUM

April 10, 11, 1979

HATFIELD POLYTECHNIC

HATFIELD

Topics: Effects of straw residues on germination pathogens, fauna.

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Professor M. A Ali
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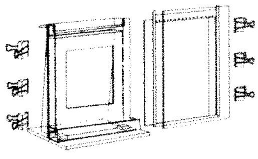

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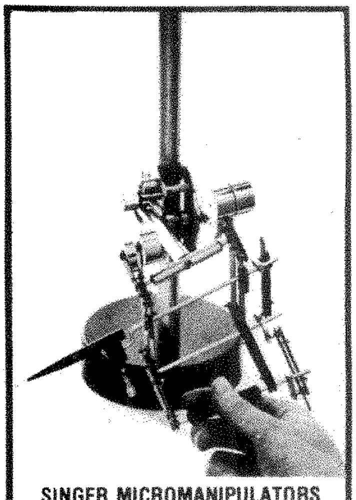

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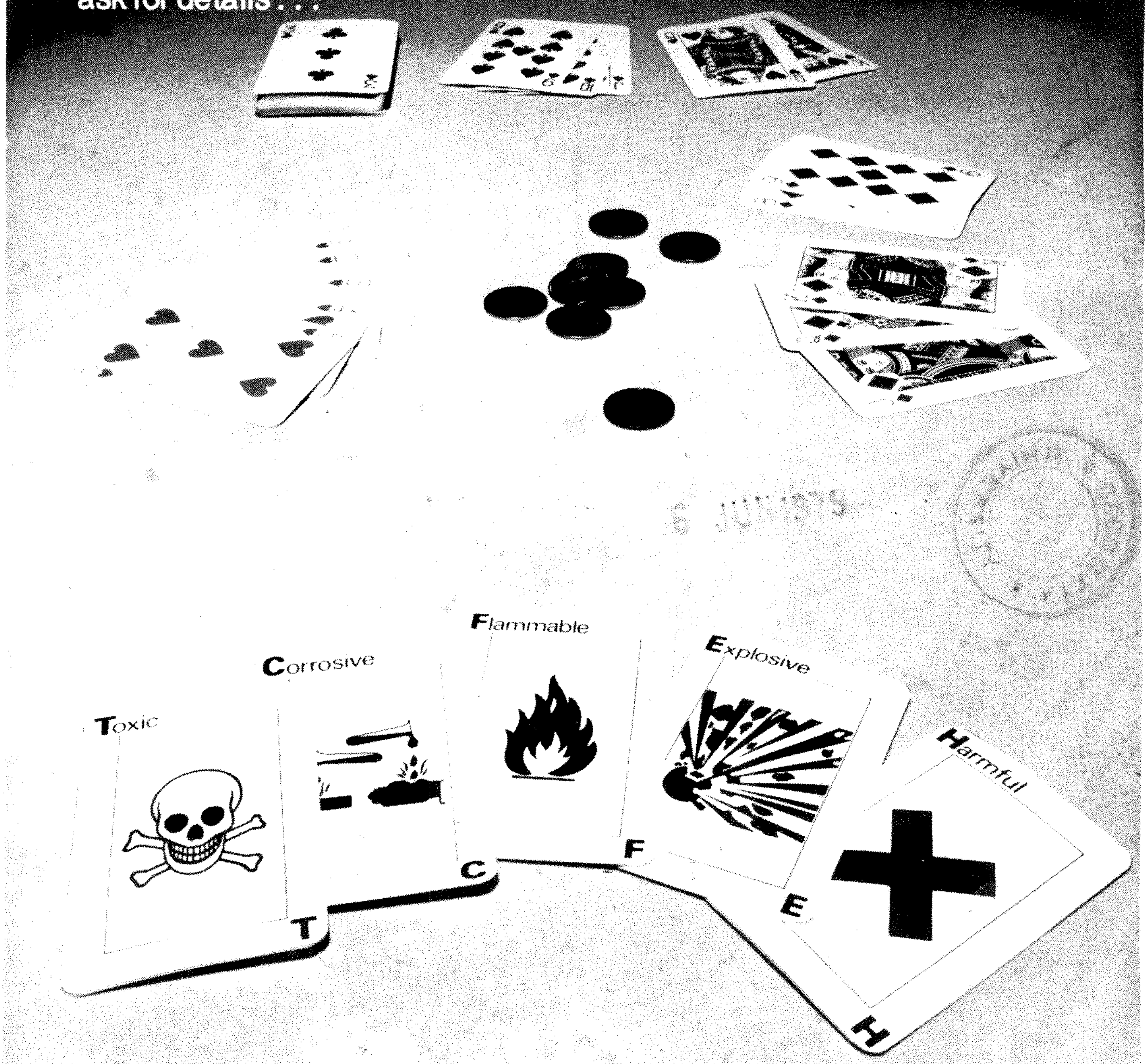

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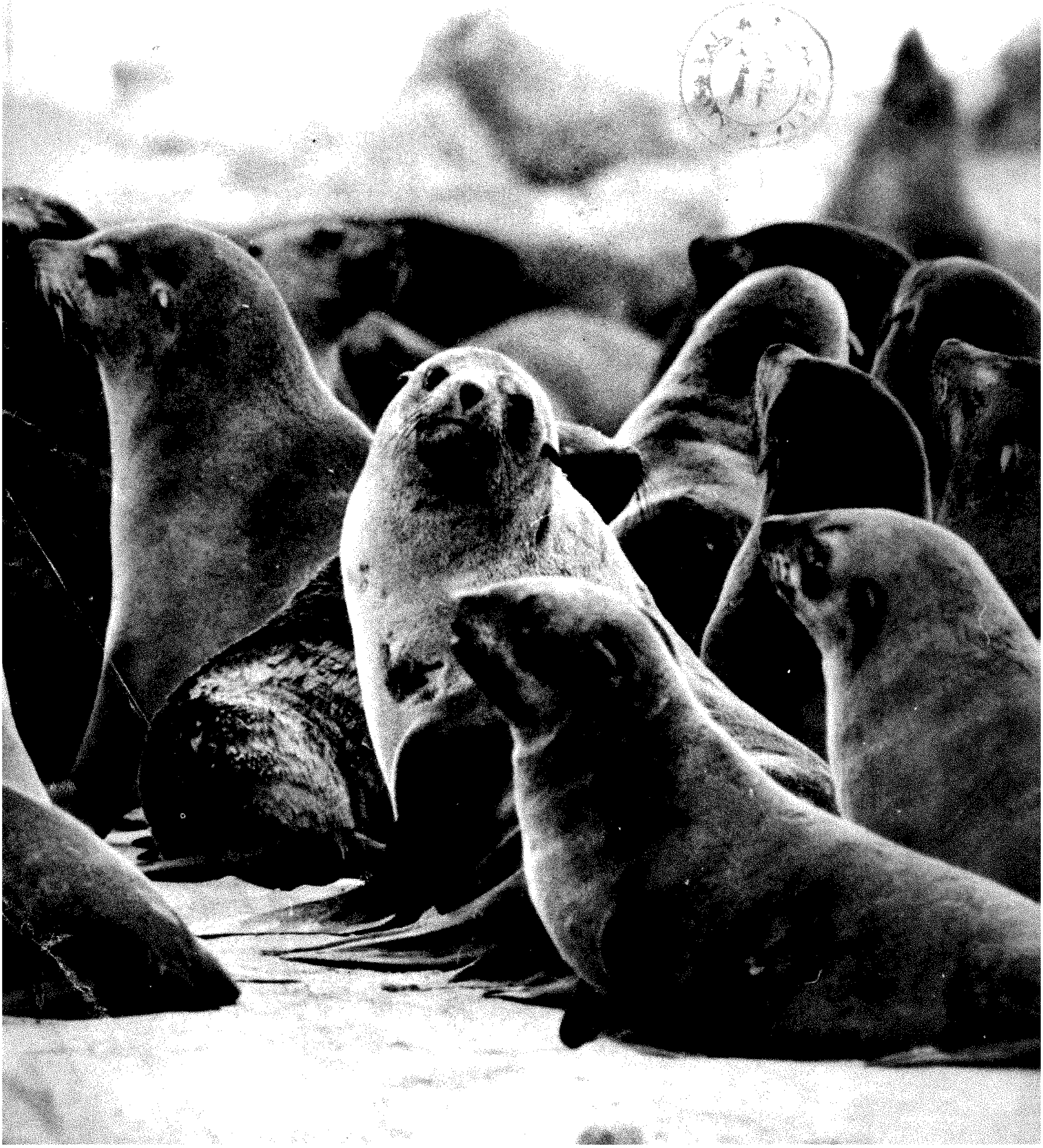

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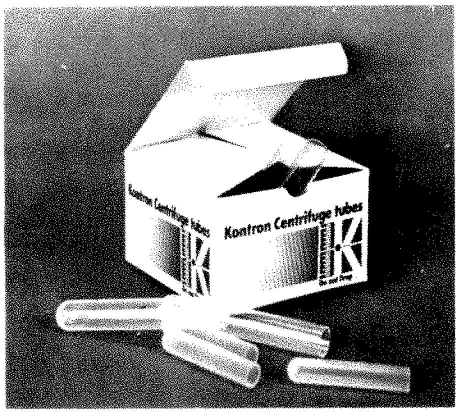

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5–15Ci/mmol NET-589

Assay of dUTPase
dUTP, [5-^{3}H(N)]-
15–25Ci/mmol NET-608

RNA Labeling in vivo
Uridine, [5,6,5′-^{3}H]-
60–75Ci/mmol NET-599

DNA Synthesis in vitro
dCTP, [5,5′-^{3}H]-
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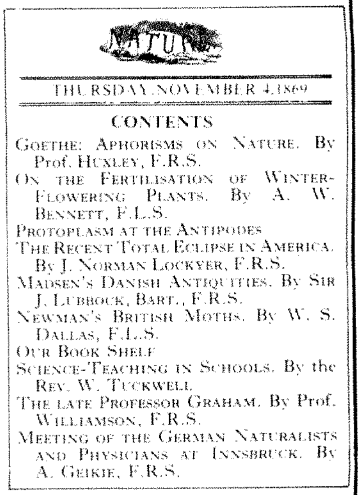

NATURE

THURSDAY, NOVEMBER 4, 1869

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Bang!—you're seriously injured

WHAT constitutes inhumane weaponry? Some, doubtless dismissed as soft-headed do-gooding pacificists, might wonder whether humanity can solve any dispute by projecting a small metal object at a velocity of up to 1 kilometre per second into the body of an adversary causing all manner of totally unpredictable damage. But so sated are we, as a culture, with letting the disagreements of individuals, groups and nations be settled by technological means totally unrelated to the merits and demerits of the case in question that the issue of just what weapons a nation can acceptably deploy has had to be faced for more than a century, and will be the subject of a United Nations conference later this year.

The St Petersburg Declaration of 1868 was the first international agreement on the subject, and its principles are worth repeating even today. They state that the only legitimate objective should be to weaken the military forces of the enemy, and for this purpose it is sufficient "to disable the greatest possible number of men". They go on to state that it would be contrary to the laws of humanity to exceed this objective by employing arms which "uselessly aggravate the sufferings of disabled men or render their death inevitable". The principles that military actions should not be indiscriminate, particularly avoiding civilians, and that weapons which cause injuries having no military utility should be banned, have continued to be at the centre of conferences ever since. In 1907 (in response to the threat of chemical warfare) the concept was added at the Second Hague Peace Conference that cruel and repulsive weapons, even with military utility, should also be prohibited. Needless to say these ideals have been widely ignored.

SIPRI, the Stockholm International Peace Research Institute, today publishes a valuable guide to the subject of inhumane warfare. *Anti-personnel Weapons* (Taylor & Francis, £9.00) by Dr Malvern Lumsden, a researcher at SIPRI, is an attempt to bring together all the scattered information available about ammunition, grenades, bombs and mines used primarily against people rather than matériel. This is done within a historical framework, making it particularly easy to see the evolution of weaponry and attitudes. Dr Lumsden also includes a brief discussion on emerging methods such as acoustic and laser techniques.

The St Petersburg Declaration was in part a response to a general concern in the mid-nineteenth century that exploding bullets might come into general use, but already by then conical bullets were inflicting more severe wounds than older spherical ammunition, and it was beginning to be noted that bullets could be designed to splay out on encountering an obstacle. Eventually, of course, this led to the Dum-Dum bullet, having a soft metal nose which expanded on penetration, causing extensive internal damage. Britain argued for the Dum-Dum bullet in colonial operations at the Hague conference of 1899, saying that whilst soldiers were incapacitated by a single projectile, "the savage, although run through two or three times, does not cease to advance"; hence the need for a more damaging bullet. But the conference reaffirmed the need to avoid superfluous injuries.

Twentieth century warfare has been somewhat different. Certainly bullets have continued to be widely used and many of these, fired from lighter weight rifles, have been of smaller calibre. This diminishes effectiveness if the bullet just drills a narrow hole through the body, but if it can be made to tumble and even fragment within the body, damage levels can be greatly raised. All current bullets are capable of causing this damage, which is reminiscent of that from the explosive bullets of the 1860s. But rifle and machine gun fire has been less successful during the First World War and subsequently at disabling the enemy so much as at containing him, up to fifty thousands rounds may be expended in modern warfare for every hit made.

In the meantime, fragmentation weapons have grown in importance until now they account for 70% of all casualties. Recent trends in bombs, grenades and shells have been to design delivery systems which disperse large numbers of small fragmentation weapons over a wide area, and to increase the number of fragments produced from individual explosions. Furthermore, weapons can be made to burst just above the ground, say at eye level.

Fragmentation weapons raise questions concerning indiscriminate use. Some can be delivered accurately but many are sufficiently widely scattered that there can be no guarantee that civilians are not equally victims of them. Delayed-action devices such as mines fall into the same indiscriminate category, often being triggered months or even years later by a non-combatant. It would be possible, however, to incorporate a neutralising device in a mine to put it out of action after a given time.

Dr Lumsden reports on these and many other devices which have been used or at least investigated, such as fuel-air explosives, concussion bombs, flechettes, toxic bullets, baton rounds, rubber bullets and so on. He describes them all dispassionately, in the best SIPRI tradition, and the book deserves to be read and thought about by a wide range of people who would not normally give serious attention to conventional warfare.

What, then, are the general questions confronting international discussions? First one must ask whether it is really possible to walk at all on the knife-edge between acceptable and unacceptable warfare. Can a line be drawn between serious injury from a tumbling bullet and death from a non-tumbling one? And can civilians and military forces really be distinguished in modern guerilla warfare? As Lumsden points out some of the problems of fair and foul fighting were faced up to during the United States' involvement in Indo-China, and the Rules of Engagement at that time deserve serious study as a basis of a way forward. Second, what is it in us that regards chemical weapons as crueller even than nuclear weapons and much crueller than guns and bombs—and is there any possibility that public opinion could in future be made to regard nuclear and conventional warfare as equally cruel? Third, is it best that we convene technical and medical conferences for precise definitions of prohibitions, a small step forward for arms control, or put all our effort into more extensive but more inaccessible measures of disarmament? ☐

US scientists to join Italians in dioxin study

An announcement is expected later this month of a joint scientific investigation into the health implications of exposure to dioxin. **Alastair Hay** reports on the protracted negotiations leading to this decision.

In February the US National Academy of Sciences (NAS) will announce the formation of a binational study group to consider the public health consequences of exposure to 2,3,7,8-tetrachlorodibenzodioxin (dioxin) Behind the decision to form the group—composed of equal numbers of Italian and American scientists—lies a history of nearly two years of discussion, negotiation and some doubt as to the value of what was seen by some as nothing more than yet another committee looking into the dioxin problem.

The initiative to form the study group was taken by the NAS shortly after the accident at Sévéso in Italy in July 1976 when dioxin from a trichlorophenol reactor was discharged over a populated area In the words of one prominent Italian scientist, the NAS wrote to the National Research Council in Italy recommending a study of Sévéso. The suggestion was couched in terms of the NAS "offering help to Italy"

It seems however, that, at that time, Italian scientists were not over-eager to conduct the study proposed by the NAS. The letter which the NAS sent went unanswered until a senior Italian scientist happened to see it by accident Recognising its potential importance he brought it to the attention of the Minister of Health urging him to reply favourably and to invite the NAS to send a team to discuss the proposition

The team, led by Norton Nelson, Professor of Toxicology at New York University, visited Italy in early April 1977.

When it returned, it made several recommendations including suggestions for developing a continuing relationship between US scientists and their Italian counterparts; the exchange of scientific and technical literature; the initiation of complementary research; and the organisation of workshops and exchange visits. It was suggested that the US programme be operated under a small steering group appointed by the NAS and its own National Research Council and that it relate to an 'appropriate counterpart' committee on the Italian side.

These recommendations were subsequently sent to Italy for approval together with a request for the names of the Italian scientists who would be participating in the programme According to the NAS the matter then 'lay dormant' until late January 1978 when a reply was received from Dr Pocchiari, Director of the Istituto Superiori Di Sanita in Rome, naming the Italian members and requesting the formation of the NAS counterpart committee

It seems to have been some months before the NAS prepared its final programme for which it sought funding The project it proposed was to be conducted in several phases, the first being simply a review of the situation Subsequently the study groups activities might include "design and evaluation of medical follow-up studies, methods, and design of systems for emergency response to similar accidents" Thus the NAS made it quite clear that it would consider a range of issues stretching beyond Sévéso Indeed, in its proposal the NAS Assembly of Life Sciences said that it would try to "develop guidelines and approaches to handling of future tragedies, and to the forecasting of potential environmental disasters".

Norton Nelson, who will be one of the US members of the study group, qualified the last proposal by saying that he is always wary of "cookbook recipes for accident prevention in future" and that he would be content if the joint programme merely provided some general guidelines for action.

With no funds of its own to implement the joint study programme, the NAS sought support from several other sources. According to an NAS spokesman the programme—which will last initially for two years—has been funded with some $120,000 by the Environmental Protection Agency, National Institute for Environmental Health Sciences and the Centre for Disease Control. Asked if F. Hoffman la Roche—the parent company which owns the reactor at Sévéso—had been approached for funds as well the spokesman insisted that it had not. One of the US study group members added that Roche would be "amongst the last we would approach" for support.

The National Academy, however, did consider asking Roche for funds, early in 1978 When informed about the NAS's suggestion to approach Roche the Italian government made it quite clear that it would have no part in the study if the company played any part in funding it. Embarrassed at having considered such an approach and anxious to avoid the cancellation of its programme the NAS had to seek its finance from alternative sources.

The NAS committee members have "not yet met", according to Nelson, but will do so in early February Following this meeting the US scientists will then meet with their Italian counterparts. "Out of that meeting we hope to formulate a programme of action", said Nelson.

Sorting out that programme could be a delicate exercise. If, as one Italian scientist put it, "it is just a NAS study in Italy, then that is very difficult and complicated On the other hand, an international study is fine" What the Italians are looking for is a clear definition of the terms of reference of the NAS study.

One of the proposals of the NAS is that an epidemiology panel be set up. But one senior Italian scientist said that although the health surveillance programme of the Sévéso residents conducted by the Lombardy Regional Council had been less than successful he was "not sure that a new epidemiological study would work"

The protracted nature of the negotiations to establish the study group has left one of the Italian group members less than enthusiastic about the proposals, particularly as the situation at Sévéso has changed so much One other criticism of the binational study group, advanced by some scientists, is that it is yet another committee set up to consider dioxin. Italian scientists say that the International Agency of Cancer (IARC) was to coordinate all information on dioxin yet that they have heard no more from the IARC

Nelson denies that there will be any "duplication of effort" He says that both the IARC and World Health Organisation know about the NAS proposals and will be kept thoroughly informed

Of one of the anxieties expressed by Italian scientists that the NAS progremme could be read as a criticism of their own activities at Sévéso, Nelson said that this should not be the case He felt that after the two week delay in evacuating the residents of Sévéso, the authorities had acted swiftly The NAS said Nelson had "no complaints about the handling of the situation by the Italians"

The binational study group has recently been described by one US scientist as a "committee of good intentions" Many other US scientists hope that it will be more than this and they seem relieved that the programme has actually got off the ground at last ☐

0028–0836/79/0277—0338$01.00

Hope for UNCSTD in New York

David Dickson reports from New York on the third meeting of the preparatory committee for the United Nations Conference on science and technology for development

WITH less than seven months to go until the United Nations Conference on Science and Technology for Development (UNCSTD) opens in Vienna the political pace of preparations is beginning to heat up with indications that the conference may not turn out quite so unproductive as some have been fearing.

The most positive indication, according to a number of delegates attending the third meeting of the conference's preparatory committee, which opened in New York last week, is a new draft preliminary programme of action prepared by the conference secretariat.

The new draft, although still felt by many to suffer a number of important omissions—such as adequate discussion of the role of women in development, or the need for appropriate technologies—is widely accepted as a major improvement on earlier versions, which had been little more than the compilation of suggestions made in national and regional papers.

In particular, the new draft contains—as the General Assembly of the United Nations has said that it should—a number of specific recommendations for action around which discussion is likely to focus in the months ahead.

Opening the committee meeting last week, Dr Joao Frank da Costa, Secretary-General of the conference said that it was often more difficult to elaborate principles than to elaborate specific action-oriented measures, and that he therefore suggested concentrating on the latter rather than the former.

In particular, Dr da Costa said it would be necessary to develop firmer guidelines on cooperation between developed and developing countries, while the developing countries themselves, for which UNCSTD was a "now or never opportunity", should investigate ways of increasing their collective self-reliance.

The preliminary draft programme of action, which will be revised following comments by delegates at the New York meeting, contains both a theoretical and conceptual framework for further actions based largely on the outcome of debates, within the UN system, on the shape of the new international economic order, as well as over one hundred concrete measures for action at all levels.

Most of the recommendations are relatively uncontroversial. They include the suggestion that the developed countries increase the amount of money they spend on research into the problems of developing countries, and that the latter should establish national science and technology policies—drawn up and executed by governments, though with the advice of broadly based national science and technology councils representing all sectors of society—and establish institutions for assessing the impact of transferred technology.

More likely to generate controversy are recommendations which would involve setting up new international funds. The draft, for example, suggests that developing countries should establish a common science and technology fund "to which the most prosperous among them should contribute more generously", and that consideration also be given to a "risk capital fund" associated with existing financial institutions within the UN system.

Equally controversial are suggestions that to go beyond the domain of science and technology to comment on more overtly political issues Paragraph 11 12, for example, suggests that in parallel to mobilising science and technology for development, "countries should introduce internal structural policies such as social reforms, redistribution of income and so on, as appropriate, so as to ensure that the application of science and technology does not result in social tensions".

How many such suggestions will survive the revision process remains to be seen. Of particular interest is whether the developing countries, through the group of 77, will be able to agree on a common set of demands. A working party established last week by the group is said to have had initial difficulty reaching a consensus But the more coherent their position, according to observers in New York, the more likely that controversial issues will stay on the UNCSTD agenda ☐

US paper stresses modernisation and meeting basic human needs

THE modernisation of developing economies, and meeting the basic human needs of developing country populations are the two over-riding objectives in applying science and technology to development, according to the US national paper for UNCSTD.

In the introduction to the paper, Father Theodore Hesburgh, previously president of Notre Dame University and chairman of the US delegation to the conference, says that these two goals are not mutually exclusive or incompatible. "The fulfilment of basic needs and the process of economic moderation can proceed simultaneously in a mutually enforcing relationship," says Father Hesburgh.

The US paper, which has gone through numerous drafts and is yet to be officially released, describes the role played by science and technology in the growth of the US economy, as well as the US's experience in applying scientific and technological expertise to the problems of development in other nations.

The paper points out that President Carter has made scientific and technological cooperation a key element of the US's relationship with other countries, particularly in the developing world. It says that the success of the developmental process in other nations is "very much in the US interests."

The paper lists recommendations for action in six areas:

- ● Improving the capacity of industrialised countries to assist developing countries upgrade their scientific and technological skills;
- ● Assisting the development of education and training programmes;
- ● Encouraging collaborative research between scientists and engineers from both developed and developing nations on global problems such as food, water and health;
- ● Helping to overcome obstacles to developing countries' access to scientific and technological information;
- ● Strengthening the links between the research communities of developed and developing nations, including joint research and problem solving;
- ● Building a more effective UN system for science and technology.

The US paper puts forward the Foundation for International Technological Cooperation, which has been proposed by President Carter, as one way in which scientists from the developed and developing nations could work together, and suggests that the UN conference "should encourage the establishment of similar institutions in other countries".

The paper also says that the many useful science and technology activities within the UN system need a central focal point and policy direction, although, it adds, not a new entity. ☐

0028–0836/79/0277—0339$01.00

Brazil bans conference on Amazon biology

The Amazon: science can help protect its environment

THE Brazilian government has refused permission for a conference on the evolution of biological systems in the Amazon region, organised by the Association for Tropical Biology, which was to have opened next week in Manaus, the region's major city.

Although no official reason for the decision has been given, scientists in the US believe that the military government may have been concerned that those attending the meeting would use it as an occasion to criticise the environmental effects of Brazil's current development policies—even though the conference itself will deal primarily with events that took place over 5,000 years ago.

The conference has been hastily rearranged to take place in Macuto, on the Venezuelan coast outside Caracas, and has received the support of the Venezuelan Ministry of the Environment. It has been convened to discuss biological models of diversification in the tropics. Speakers will address topics such as the relationship between climatic change and species differentiation in the Amazon and its surrounding savannah region.

All the original speakers—including a number from Brazil—are expected to attend as planned. However, the change of location may prevent the participation of some Brazilian research workers and students, for whom lengthy visa negotiations, as well as the deposit of a large sum of money, are necessary for foreign private travel.

"This conference will bring together specialists in disciplines ranging from archaeology to entomology and genetics. Everything is now working together, and we are coming up with some exciting material, such as the correlation between archaeological sites and past climatic changes," Dr Clifford Evans, curator of South American archaeology at the Smithsonian Institution in Washington, DC, and one of the main organisers of the conference, said last week.

The military government's decision, which was taken on the advice of the National Security Council, has provoked a storm of protest in the Brazilian press, from which censorship has only recently been lifted. Critics, including a number of eminent scientists, have accused the government of interfering with academic freedom, and of ignoring how science is necessary to preserve the environmental integrity of the Amazon region.

David Dickson

Second thoughts on study of nuclear risk

THE Boston-based Union of Concerned Scientists has called for the temporary shutdown of 16 US nuclear power plants for safety modifications, in the light of evidence that previous estimates of "low probability" risks may have been unjustified.

This demand follows the decision of the Nuclear Regulatory Commission to withdraw its support from important parts of a study published by the commission three years ago on the safety of nuclear reactors, which indicated that the risks from nuclear power were much less than from other man-made activities.

The study was carried out for the NRC by a team headed by Professor Norman Rasmussen, of the Massachusetts Institute of Technology. On its publication in 1975, the NRC described the report as a "soundly based and impressive work".

The conclusions of what has become widely known as the Rasmussen Report have been frequently used by supporters of nuclear power to defend existing and proposed programmes. However, criticism by others of the report's methodology and its conclusions—in particular by Congressman Morris Udall, chairman of the House Committee on Interior and Insular Affairs—led the NRC to appoint a review group headed by Professor Harold Lewis of the University of California, Santa Barbara.

In its report, which was presented to the commission in September, the review groups said that it found the methodology used in the study—based on a fault-tree/event-tree form of analysis—to be basically sound. However it had a number of reservations about the way that the results of the analysis had been presented.

In particular, the committee criticised the narrow limits which the Rasmussen report had placed on estimates of the probability of a melt-down of the reactor core. The reasons for understating the error band, said the committee, included an inadequate data base, a poor statistical treatment, and an inconsistent propagation of uncertainties throughout the calculation.

The review committee also criticised the executive summary of the report—the part which has been most widely quoted in public discussions. The committee says that the summary did not sufficiently emphasise the uncertainties involved in calculating the probability of risk and that this might have led readers to a "misplaced confidence" in the validity of the risk estimated.

In a statement issued last week, the NRC commissioners say that they accept the criticisms of the review committee, and as a result have withdrawn any explicit or implicit endorsement of the report's executive summary.

On accident probabilities, the NRC says that the absolute values of risk in the Rasmussen report "should not be used uncritically either in the regulatory process or for public policy purposes". (The report states, for example, that nuclear power plants were about 10,000 times less likely to produce fatal accidents than man-made non-nuclear activities.)

The NRC's decision to support the Lewis Review Committee's findings has prompted the 65,000 strong Union of Concerned Scientists, which has been among Rasmussen's most vocal and persistent critics, to demand additional safety precautions at 16 plants where, they claim, there is evidence that the report was used to justify the acceptance of current safety procedures.

"Although the risks are still indeterminate, the uncertainty band includes an area of risk which is unacceptable, and it is on this basis that we are asking for the shutdowns", Dr Henry Kendall, Professor of Physics at Massachusetts Institute of Technology, said last week.

David Dickson

HSE confident it can cope

THE UK Health and Safety Executive (HSE) is confident that it is adequately staffed to inspect all UK laboratories doing genetic engineering experiments.

In reply to questions by the House of Commons Select Committee on Science and Technology last week, the HSE said that its three specialist inspectors for microbiological research could cope with inspecting the UK's seven Category Three and two Category Four laboratories. The time lag between notification of a laboratory's intent to conduct Category Three or Four experiments and inspection had never exceeded six weeks.

Laboratories conducting Category One and Two experiments do not need to be inspected before work can commence The HSE felt that because the inspection of these laboratories does not require such specialist knowledge, regional inspectors of medical laboratories could cope with them So far, about 200 experiments in 50 centres have been notified to the Genetic Manipulation Advisory Group (GMAG). Since last July the HSE and GMAG have jointly inspected 14 of the centres.

GMAG has recently had a change of membership Seven new members, J Chamberlain, Sir Frederick Dainton, J. Ingle, M Kogan, C. M Puxon, P. M. B. Walker and P. Wildy together with a new chairman Sir William Henderson started work in January Under its new chairmanship, GMAG may be taking a more positive attitude to work with recombinant DNA. Sir William has few fears about the potential scale-up of the processes in industry. He told the select committee at a previous meeting that "the hazards of scale-up are very much less than has been anticipated". He believes that 4,500 litres of reagent in clean, sealed stainless steel flasks are probably safer than the intermediate stage· "the danger is when you move from the bench to the 5 or 10 litre scale, when you don't have adequate procedures"

GMAG is setting up a subcommittee on scale-up, and Sir William is playing close attention to its membership He feels it should have a strong representation of chemical engineers who have experience of risk assessment in industry.

Dr Ken Duncan of the HSE shares Sir William's lack of fear about the dangers of scale-up to industrial processes when this happens, he says, the HSE will be able to call on its chemical engineers, currently engaged in other activities within the HSE, for advice Duncan, however, does not foresee unusual problems with scale-up. ☐

UK tightens up on lab safety

MANY OF the recommendations of the Shooter report, which called for tighter laboratory safety regulations following the smallpox outbreak at Birmingham University, are now to be implemented by the Government The move follows the unofficial "leaking" of the report by Clive Jenkins, general secretary of the Association of Scientific, Technical and Managerial Staffs. The Government's decision reveals that it no longer considers the country's voluntary safeguards for handling viruses in laboratories as adequate.

Social Services Secretary David Ennals told the House of Commons last week that several changes were to be made These include:

- Regulations compelling laboratories to give notice of their proposed work.
- An official licensing system to replace the present voluntary method of approving category A pathogen laboratories
- Accepting the recommendation that category A laboratories should be inspected annually.
- Setting-up a review of the Dangerous Pathogens Advisory Group, the body responsible for approving these laboratories, to find ways of broadening its membership to include wider union and public interest

No new legislation will be introduced to carry out these measures, which will be implemented as part of the Health and Safety at Work Act. Mr Ennals said public fears following the smallpox outbreak that killed medical photographer Janet Parker last September should not be allowed to cause panic If overprotective measures were introduced, there could be great damage to the ability to carry out effective diagnosis or research.

Mr Ennals also revealed that a reinspection of existing category A pathogen laboratories—which handle smallpox, rabies, lassa fever and other dangerous viruses—is now being carried out by the Health and Safety Executive. "In some cases it has become clear that improvements in safety procedures are necessary and immediate action has been taken", he said.

Details of these cases of "immediate action" were revealed by the HSE last week and consisted of stopping work with category A pathogens at two laboratories. The first, the public health laboratory at Colindale, North London, the only centre for rabies diagnosis in Britain, was visited on January 18 by HSE inspectors and a request for an end to dangerous virus work was issued

The order was made in the form of a Crown notice, which is not legally enforceable as one Government body cannot prosecute another, although the HSE has recently called Mr Ennals to remove Crown immunity for such laboratories. However, the director of Colindale, Dr Eric Mitchell, said a new purpose-built virus laboratoriy was to be opened there in a few weeks—after it had been inspected by the HSE, DPAG and the Home Office.

The other laboratory, at the Lister Institution in Elstree, Hertfordshire, has been asked to improve safety precautions in the production of rabies vaccines The laboratory was closed by the institution last June, and the HSE has instructed that it must not re-open without its approval.

Robin McKie

Switzerland to consider revised guidelines

Two months ago Mrs Shirley Williams, Secretary of State for Education and Science speaking on the television programme *Weekend World*, said that she would like to see the establishment of some form of international guidelines to regulate research on recombinant DNA Commenting on the different guidelines already in operation in the United States, Britain and the European Community, Mrs Williams singled out Switzerland as one country operating outside these formal rules. There was a need, she said, to induce it to come within some form of "containment barrier".

Switzerland is of course the home of the large multinational pharmaceutical companies F Hoffmann–La Roche, Sandoz and Ciba-Geigy. Roche and Ciba are both aware of the commercial possibilities of genetic engineering—and both have a finger in the pie Roche sponsors the Basle Institute for Immunology and Ciba the Friedrich Miescher Institute, also in Basle Both institutes have begun recombinant DNA research which is, as yet of a fundamental, rather than an applied nature.

But all recombinant DNA research in Switzerland whether commercially, privately or publicly sponsored is subject to certain controls The controlling agency is a commission set up by the Swiss Academy of Medical Sciences in 1975 In an open letter dated April 1978, the commission set out its control policy. It was the commission's view that the U S National Institutes of Health (NIH) guidelines should operate in Switzerland and it recommended this to all practitioners in the field. The commission also made it clear that the responsibility for the safety of this work lies with the principal investigator.

Professor Werner Arber of the Biozentrum at the University of Basle, and one of the 1978 Nobel Laureates in Medicine is a member of the commission. Commenting on the responsibility of the investigator, he told *Nature* that. "It is perhaps important to say that the Swiss Law of Epidemics foresees that any work with pathogens and their products has to be carried out with the maximum of care, and our commission interprets this formulation in such a way that recombinant DNA [research] done in our country has also to be done with a maximum of care".

The commission compiles an annual register of all work done in genetic engineering. In 1978 there were some 31 projects in the field under 18 principal investigators, eight of whom were based in Basle. The projects are divided into two groups according to their source of DNA for recombination. Category 'a' experiments are those using cellular DNA and using *E coli* as host; and category 'b' includes those using DNA from plasmids, bacteriophage and viruses and again using *E coli* as the host. Twenty-two of the projects are in category 'a' and include work with embryological tissue from primates, other mammals, birds and cold blooded vertebrates. Eight of the 'a' category are listed as using 'inferior eukaryotes' as a source of DNA. In category 'b' the recombination work is listed as involving plant viruses, 99% pure organelle DNA from eukaryotes (but not primates) and plasmids or phage DNA from hosts, the genes of which are interchangeable with *E. coli*.

Of the 18 principal investigators 15 are based in universities, with the remaining three privately sponsored.

It is the European Science Foundation (ESF) which is trying to harmonise the guidelines and working conditions in recombinant DNA research in Europe. Arber represents the Swiss Commission at the ESF meetings and appreciates the effort the Foundation is making to find the common ground. However, Arber says that the Swiss Commission has still not reached a firm decision on the guidelines it will adopt in the future. In the next few months the commission will meet again to consider the revised NIH guidelines (which lift restrictions on some categories of recombinant DNA research, but which for the first time extend the controls to cover industry), those of the UK Genetic Manipulation Advisory Group, and the guidelines proposed for France and Germany.

Alastair Hay

Catch a falling Kosmos—and send the bill to Moscow

OPERATION Morninglight—the clean-up of northern Canada, following the "unscheduled re-entry" and crash of the Soviet satellite Kosmos-954 a year ago is now over, at a cost of some $13 million. Canada is sending a bill for $6 million to the Soviet Union.

Kosmos-954, which carried a power-source fuelled with some 50 kg of enriched uranium, crashed in the area of the Great Slave Lake on 24 January 1978. The first stage of Morninglight was launched within hours—a massive air search for radiation hot-spots. Within four days, three radiation hot-spots were located, and several more within the next week.

A party of six meteorologists encamped in the Warden's Grove area, two of whom actually touched a fallen fragment, were evacuated to Edmonton for radiation tests; but they showed no signs of contamination. As for the local Chipewyan Indian population, apart from a missing pair of trappers who were feared to be camping near a hot-spot, the greatest danger seemed to be fear and bewilderment as to the nature of the search, plus the ominous appearance, in the settlement at Snowdrift, of a wolf which refused to be driven away and finally had to be shot.

The second stage of Morninglight—a ground level survey—was scheduled for July, when the snows had melted. Shortly before it was to begin, uranium prospectors reported satellite debris in northern Saskatchewan, and the search was extended beyond the Great Slave Lake area. Morninglight finally covered some 90,000 square kilometres, from which over 3,000 pieces were recovered, mostly small particles, but with a few larger fragments, one of which was said to be—"as big as a five gallon drum". One of the larger pieces was so radioactive that a special lead container had to be built by the University of Alberta. Another fragment, though, a 225 cm × 50 cm stove-pipe shaped cylinder, was sufficiently "cool" to be loaned for temporary exhibition to the National Museum of Science and Technology in Ottawa.

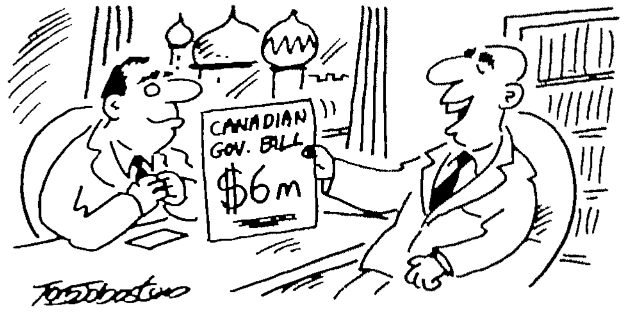
By the beginning of winter, when phase two of Morninglight ended, the Atomic Energy Board of Canada announced that the residual radiation risk in the impact area was minimal. Any remaining radioactive debris would soon decay to below natural background level, and the hazard to humans or wild life from accidentally ingested particles would be no greater than that from a medical x-ray examination. Nevertheless, as an additional precaution, the Federal Environment Department would monitor fish from the Great Slave Lake, while the Health Department would check the meat of migrating caribou herds.

Apart from these minor hazards, and the $13 million bill, what are the lasting effects of the Kosmos-954 incident? First, the setting up, on Canada's initiative, of a UN working group to study the technical aspects and safeguards for nuclear power sources in space, the first meeting of which will take place later this year. Minor spin-offs include a considerable amount of practice in the aerial and ground location of radioactive debris, and the (at least temporary) appearance on the map of the name "Cosmos Lake" to denote the base camp of the aerial search.

Canada's claim against the Soviet Union will doubtless provide international lawyers with fascinating material on compensation norms and procedures. As far as the average Canadian is concerned, there remains a profound sense of thankfulness that the impact took place in a virtually uninhabited area—and considerable apprehension of what could happen next time.

According to NASA officials, there is a 2% chance of debris from Skylab striking Canada. Moreover, any such impact would occur south of latitude 50°N—a band which includes all major Canadian cities except Edmonton and Calgary. Not surprisingly, Canadians are said to find the news of the forthcoming descent of Skylab to be, at the mildest, "somewhat disturbing".

Vera Rich



"There's a fair chance that the Skylab crash will wipe out all record of this bill, comrade!"

news in brief

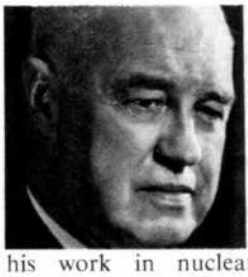
ASTMS branch wins redundancy rights: A local branch of the Association of Scientific Technical and Managerial Staffs has persuaded the Royal Free Hospital School of Medicine to withdraw waiver clauses concerning redundancy rights from its fixed term contracts. The school secretary, Mr G. W. Fenn, refused the initial ASTMS request. Negotiations were then referred to the school's joint negotiating committee, composed of three ASTMS representatives and three administration representatives. They also rejected the proposal but after discussion agreed to present the request to the full school council (from which ASTMS representatives are excluded). The council agreed to withdraw the part of the waiver clause requiring employees on fixed term contracts to give up their rights to redundancy pay but have retained the waiver for rights against unfair dismissal. "To maintain happy relations in a small organisation like ours, we felt it was a good thing to do for the employees," said Fenn.

UNEP calls key meeting on Mediterranean pollution: The United Nations Environmental Programme is convening a meeting of the Mediterranean Action Programme from 5–10 February to discuss the activities of the 1979–1980 MAP programme, the location of MAP headquarters and most important, the share out of \$2¼ million operating expenses among the 17 member states. Some struggle is expected about the exact terms of the share out but the conference is expected to make slow steady progress towards the eventual goal of a full Mediterranean clean-up. The major problem, towards which the conference will make a small contribution, is land-based pollution—industrial water, domestic sewage, agricultural pesticides and fertilisers—which account for 85% of Mediterranean pollution. The World Health Organisation estimates that it will cost \$5 billion over a period of 10 to 20 years to control pollution at the source, often hundreds of kilometres upstream from the Mediterranean.

US prepares for Skylab re-entry: The US government has set up an interagency group to prepare for possible damage when Skylab re-enters the earth's atmosphere later this year, Dr Robert Frosch, administrator of the National Aeronautics and Space Administration, told a Senate panel last week. The State Department, the Department of Defense and the New Federal Emergency Management Agency are working with NASA to set up an international warning system and an assistance team to deal with any damage or injury caused by debris from the 80-ton space station as it re-enters the atmosphere.

Dr Frosch told the Senate subcommittee on space and science: "There are potential options of exerting some influence on what happens to Skylab during its last couple of orbits." But, he said, NASA scientists did not yet know how much control they would have over Skylab's break-up —and in particular whether they could bring it down over an ocean. One critical factor was the power supply, and whether Skylab's solar panels could be kept fully charged. If Skylab could not be reliably brought down into the sea, it might be wise not to try to influence its descent, Dr Frosch suggested, because of the political implication of the US trying to crash the debris into a particular part of the globe.

Sullom Voe oil spill: Census taken by the Royal Society for the Protection of Birds after the Shetland Island oil spill, where 1,150 tonnes of heavy bunker fuel oil fouled fifteen miles of coastline has shown the spill to be "locally disastrous" according to Bobby Tulloch, RSPB representative. 2,800 birds from 45 species have been killed. Of the local eider population of 350 birds, 5 are left, with a similar picture extending to other species. Twenty others have been killed or "badly affected" and it is not clear whether any remain. Local sheep that graze on seaweed deposited along the rocky coast have become polluted with unknown toxic and carcinogenic effects.



Soviet award for Todd: The Lomonosov medals, the highest awards of the Soviet Academy of Sciences, are awarded each year to one Soviet and one foreign scientist. The 1978 awards, announced last week, go to Academician Anatolii P. Aleksandrov, president of the Soviet Academy of Sciences, for his work in nuclear physics, and Lord Todd (left), president of the Royal Society, for his work in biology. The linking of the two Presidents in this way is something of an auspicious coincidence. The Royal Society has a long standing scientific exchange programme with the Soviet Academy, dating back to 1956 and the very beginning of the post-Stalin "thaw". The then Dr Alexander Todd helped establish this exchange.

British students swing to science: Statistics from the two major school examination boards in Britain are showing a trend back towards physical sciences at A-level, at the expense of subjects like history and geography. Recovery from the slump in interest of the mid-1970s is particularly apparent in chemistry, where the number of candidates entering the London Schools Examination Board A-level has increased from 14% to 16% in the last four years, while the Joint Matriculation Board's chemistry candidates accounted for 21.3% of total entries, the highest proportion since their comparative statistics began in 1971. Physics and mathematics are following the same trend, with increases of around 1% to 2% over the past two to three years. With a total A-level entry to these boards of 66,000 and 55,000 respectively in 1978, even a small percentage increase indicates a significant swing towards science.

However, Professor L. A. B. Elton of the Institute for Educational Technology, Surrey University, considers the statistics as presented are "too bland for anyone to draw sensible conclusions". They do not distinguish between male and female candidates, for example.

Seasat failure due to technical and management faults: The failure of the Seasat satellite, which was launched early last year by NASA and which ceased transmitting data in October, was the result of a "massive and progressive" short circuit, due to a "lack of proper attention" by NASA's jet propulsion laboratory and the Lockheed Missile and Space Company to testing of the rocket stage to which the satellite was attached. "As a result, a test was waived without proper approval, important component failures were not reported to project management, and flight controllers were inadequately prepared for their task," said a board of enquiry in a report issued last week.

Seasat was launched to test equipment for gathering data on the world's oceans for both scientific and meteorological purposes. Before the failure occurred the satellite had been transmitting data for 99 days. The report says that the most likely cause was an electrical arc between adjacent slip ring brush assemblies.

Correction: President Carter has requested a budget increase for basic science of 2% above inflation, not 20% as reported on page 252 last week. . . . alas.

Does the UK have a numeracy problem?

Joseph Schwartz puts his own view of the controversy over the mathematical abilities of UK schoolchildren

IN March 1978 Dame Kathleen Ollerenshaw, President of the Institute of Mathematics, released her account of innumeracy in Great Britain to the press. "Simple sums floor vast majority of pupils" was the *Times* headline. An examination administered to 7,000 15-year-olds in six education districts produced "abysmal" results.

The test consisted of 17 questions, of which nine had two parts and two had three parts, requiring a total of 44 basic operations to be performed in 40 minutes. The problems ranged from 12 computations of the type $14+35=?$ to nine word problems (How much do I save if I buy two large bottles of shampoo (150 cc @ 25p) instead of taking the same quantity in small bottles (75 cc @ 15p)?) and included questions involving the reading of graphs and train timetables.

Calling a mark of 90% adequate and a score of 80% well below an acceptable standard, Institute members were disappointed in the actual results. The percentage of pupils receiving an 80% score in the six districts ranged from 21% to 61%. The percentage of perfect papers ranged from 1% to 13%. Dame Kathleen said "education as a whole, and parents and employers will agree, is not doing a good enough job".

More recently, in September 1978, the Department of Education and Science announced the formation of a Committee of Inquiry into the teaching of mathematics in schools. This new committee is a consequence of the 1976-1977 Tenth Report of the Expenditure Committee on the Attainments of the School Leaver in which mathematics was particularly singled out for attention. "We heard more statements about mathematical competence or lack of it than about any other school subject. The CBI described it as the issue most frequently mentioned by their members".

Dr H R Pitt, a mathematician and now Vice-Chancellor of Reading University, testified "I think there is no doubt that there is something of a crisis in the teaching of mathematics", attributing the crisis to the introduction of "a lot" of new methods, too rapid change of curriculum and a shortage of skilled teachers. The terms of reference of the Committee of Inquiry are: "To consider the teaching of mathematics in primary and secondary schools in England and Wales with particular regard to the mathematics required in further and higher education, employment and adult life generally and to make recommendations".

But workers in the field of numeracy and maths instruction greet these developments unenthusiastically. Jo Stephens of the DES Mathematics Inspectorate points to some historical antecedents of the present upsurge of interest in mathematics.

In 1954 the Mathematical Association reported: "The standard of mathematical ability of entrants to the Trade Courses is often very low".

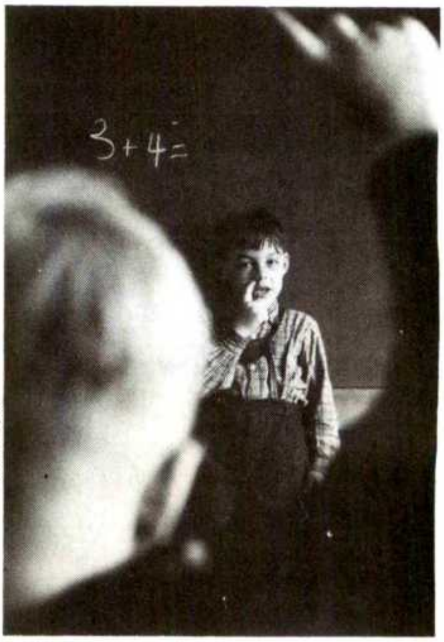
In 1925, the Board of Education reported: "It has been said that accuracy in the manipulation of figures does not reach the same standard which was reached 20 years ago. Some employers express surprise and concern at the inability of young persons to perform simple operations involved in business".

In 1912 an LCC report noted: "There is a prevailing opinion that London elementary school children of today are slower and less accurate in computation than they were ten years ago". And in 1876 the Mathematics Inspector reported: "In arithmetic I regret to say worse results than ever before have been obtained—this is partly attributable no doubt to my having framed my sums as to require more intelligence than before . . .".

The problem, then, is not a new one. Seen in this light the current focus on numeracy is simply a political football to be pursued by the media and government as it suits them.

Blanket blame of teachers, attacks on mixed ability classes and an abstract concern with 'standards' characterise the debate so far. Some members of the Expenditure Committee are alive to the fact that a person's numerical skills bear little relationship to the requirements of the available jobs. "Unqualified school leavers competing for the diminishing pool of jobs at below craft level find that they lose out to the qualified school leaver who is eager to take a job below his (sic) capability rather than be unemployed".

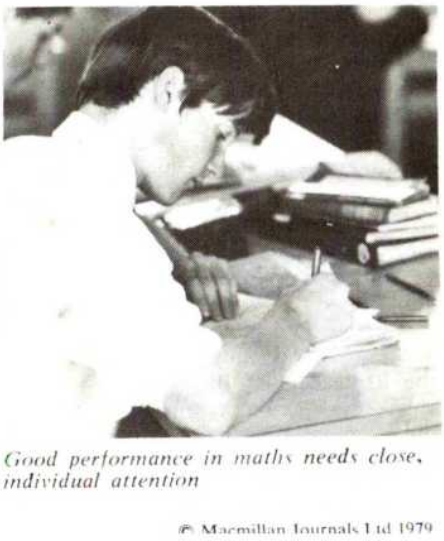
In other words, in a buyer's market, employers are selecting the qualified school leaver for jobs that would normally go to the unqualified leaver. The unemployment problem is not due to a lack of competence of the workforce. Making people more numerate may help them to compete more effectively but it will not increase the number of jobs. To imply that it will, however indirectly, is to make a scapegoat out of the schools.



Barnaby's

Nevertheless, Dame Kathleen's report does indicate that there is room for improvement in the mathematical abilities of school leavers. Where *does* the problem lie? The teachers themselves should surely know best.

Most numeracy teaching is based in fifteen Colleges of Further and Higher Education in the London area. The problems presented to teachers are extremely varied—as are the students. A major facet is that the problems of numeracy and literacy are intertwined. There are very few people who can work with numbers without being able to read and as such many numeracy problems are handled within the much larger literacy programmes. The majority of remaining students are young adults who either have been referred to numeracy courses from programmes like Manpower Services Commission's Training Opportunities Scheme or who feel the need to improve their computation skills for various courses ranging



Barnaby's

Good performance in maths needs close, individual attention

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from craft apprenticeships to office work.

Linda Major, an Advisory Teacher in numeracy based at Westminster College, says that students generally have little trouble with addition but do have certain problems with other operations. Frequently, she says, students will be able to use subtraction in the act of handling money but will have difficulty in working it out on paper. Major feels that some part of the traditional curriculum such as long division and fractions are of little practical use in adult life and makes no attempt to teach this.

Relevance is crucial to the approach. The students are riveted when it comes to the question of tipping but it is another matter when percentages are taught. (Literacy methods are similar. Jud Stone of City and East London College uses materials created by the students themselves. Instead of reading texts of little immediacy students learn to read their own words. The method places far more emphasis on writing than reading.) Major says that the goal of fluent single digit addition and multiplication along with a knowledge of how to compute with larger numbers using the ordinary place value algorithm is attained when there is adequate staffing.

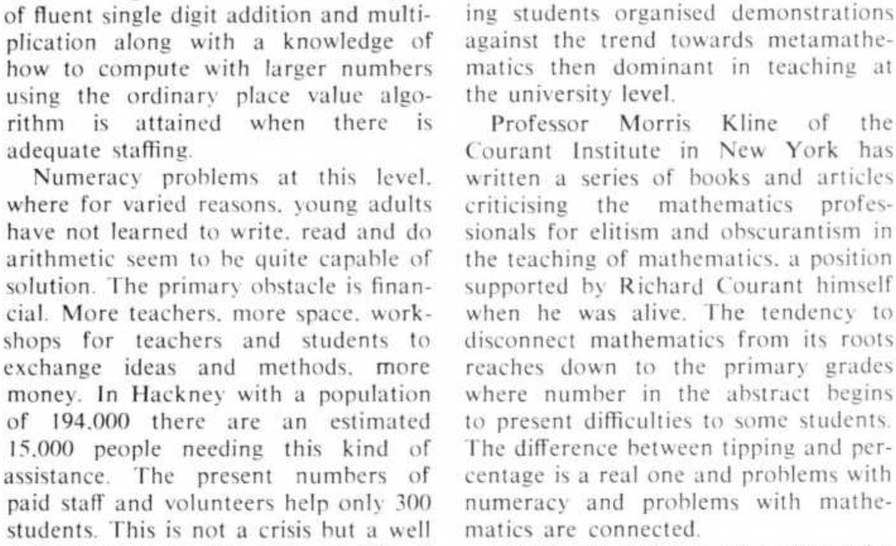
Numeracy problems at this level, where for varied reasons, young adults have not learned to write, read and do arithmetic seem to be quite capable of solution. The primary obstacle is financial. More teachers, more space, workshops for teachers and students to exchange ideas and methods, more money. In Hackney with a population of 194,000 there are an estimated 15,000 people needing this kind of assistance. The present numbers of paid staff and volunteers help only 300 students. This is not a crisis but a well defined need on the part of 8% of the population of Hackney. Current expenditure is too low. The Adult Literacy Support Services promises some additional volunteer assistance but it is doubtful that without proper training, proper space, proper materials, in short, proper organisation, that such an intervention can be effective. Some workers question the wisdom of basing a programme on unpaid labour but in the current situation they are not going to refuse help in any form.

At a higher level, more fundamental questions can be raised. University graduates in general and scientists in particular have all experienced difficulties when confronted with mathematics. According to Emilio Segrè, Enrico Fermi attended a conference at Bohr's institute on the weak interactions. At the end of two hours of symbols the lecturer concluded, "And that is Fermi's theory of the weak interaction". Said Fermi, "It was the only part of the lecture I understood".

Mathematics has had in its history a long dishonourable thread of mystification and inaccessibility going back to Plato. The most militant opposition to this tendency that I know occurred in Germany in the 1890s where engineering students organised demonstrations against the trend towards metamathematics then dominant in teaching at the university level.

Professor Morris Kline of the Courant Institute in New York has written a series of books and articles criticising the mathematics professionals for elitism and obscurantism in the teaching of mathematics, a position supported by Richard Courant himself when he was alive. The tendency to disconnect mathematics from its roots reaches down to the primary grades where number in the abstract begins to present difficulties to some students. The difference between tipping and percentage is a real one and problems with numeracy and problems with mathematics are connected.

Additional criticism of mathematics along these lines is starting to come out of the analyses of sex differences in educational achievement. Alison Kelly, a lecturer in sociology at Manchester University says that as well as social pressures in all their forms that are exerted on girls to avoid science and maths there is the problem of the stereotypical image of science and maths itself—cold, remote and abstract.



Teaching relevancy at an early age

Anthea Sieveking

Kelly and other analysts assert that women's rejection of mathematics is far more active than the usual portrayal of women as passive victims of a social role. "Women reject mathematics and the sciences as military and industrial activities bearing no relationship to helping activities or being able to explain oneself to oneself". If this is true of large numbers of women it is interesting to speculate on the possible effects of a major government effort to recruit more women to engineering, maths and science.

A different approach to the well-educated person's problems with mathematics was tried last year by Larry Buxton of the DES Maths Inspectorate. Recognising that there were important emotional responses to mathematics Buxton attempted to deal with these directly using the methods of psychotherapy He worked with a group of six adults for 24 sessions and with three individuals for 12, 24 and 36 sessions respectively.

On the basis of the experience Buxton is convinced that the central problem is as much emotional as cognitive. "The exploration of emotion is not supposed to be related to in mathematics yet it is the subject with the most charged emotional content". Numeracy teachers deal routinely with problems on this level although it seems unlikely that widespread intervention based on individual counselling is feasible. But Buxton thinks that two widely accepted practices in mathematics instruction are open to question.

The first is the use of timed tests. The present curriculum confuses the need to work hard with the need to work fast, he says. Good performance in mathematics needs close, concentrated undivided attention and students must not feel rushed. The second practice involves complex attitudes towards authority. An interrogative approach in the classroom based on categorical right or wrong answers can be threatening and off putting to many students. This combination of time and authority was a recurring theme for the people with whom Buxton worked.

So there are many directions in which the teaching of mathematics can be improved. But a barnstorming attitude based on the false perception of a 'crisis' and on weak analysis of the problem is going to achieve very little. If the government wishes to improve the mathematical level of its schoolchildren it could do a lot worse than to listen to those teachers most directly confronted with the problem. □

correspondence

GMAG should look to NIH

SIR,—I write to enquire whatever happened to the public debate on changes in the regulations for genetic manipulation experiments? The notion of rationalising genetic manipulation containment by measuring the risk in each experiment was put forward in the columns of *Nature* last month. A public meeting was promised and was indeed convened in London on 21 December. This meeting could be called neither public nor a debate and achieved little or nothing. Views were expressed by members of GMAG, both on the panel and in the audience, that revealed strong political polarisation but little in the way of scientific evidence supporting either the present guidelines or possible alternatives. From the body of the meeting the only view which came across strongly and repeatedly was the indignation of many scientists at the classification of experiments involving the manipulation of *E. coli* DNA in *E. coli*. A host of important questions concerning the proposed new systems of evaluating the necessary containment for particular genetic manipulation experiments were not raised at this meeting. Even where one or two points were raised, time for discussion was inadequate. It is safe to conclude that there has been no proper forum for public debate.

By contrast to events in this country we have the example of the way in which the NIH guidelines have recently been revised in the US. Irrespective of whether one agrees or disagrees with the new NIH guidelines one must admire the broadly based public debate, the informed political comment and the rapidity of the process of modification of the proposals in response to debate and comment. Moreover the whole process, up to and including publication of the new guidelines, is freely available in the Federal Register. We could, to our advantage, learn from those events.

The need to change the genetic manipulation guidelines in Britain is just as pressing as was the need in the US. Our present guidelines are illogically restrictive. The levels of containment imposed upon genetic manipulation experiments in Britain have from the beginning exceeded those required in the US. This difference taken together with the lack of availability of physical containment facilities in this country has meant a severe handicap for British work with systems designated as requiring high containment. The introduction of the new NIH guidelines has widened the gap between containment required in the US and that required in Britain to such an extent that it is now impractical to continue a wide range of genetic manipulation experiments in this country. On a global basis it perhaps matters not whether Britain is involved in genetic manipulation research. For Britain however, the consequences of missing out on a revolution in biological technology and in losing a generation of the best young scientists who wish to be trained in that area will be dire.

If the guidelines for genetic manipulation experiments in Britain are to be changed then the change must be effected rapidly. There is no sign of swift action on the Brenner initiative nor of an informed debate. Looking again at the global picture, should we be so insular as to think that we should repeat a process which has been painstakingly conducted in the US. Biological risks by their very nature do not respect national boundaries. We would be wise to look carefully at the guidelines accepted by the majority of scientists working with genetic manipulation and by the nation within which they carry out their work. I think that we could, without endangering either workers in the field or the public at large, adopt the philosophy of the NIH guidelines and indeed, with certain modifications to British conditions, the letter of those guidelines. The NIH guidelines remain largely empirical but they are based on extensive experience. In science we do not look at national origins in evaluating ideas or experimental results. I trust that in this case that neither national pride nor political ideology will prevent serious consideration of the NIH guidelines as a suitable model for genetic manipulation work in Britain.

Yours faithfully,

ALAN R. WILLIAMSON

Institute of Biochemistry,
University of Glasgow, UK.

The status of safety officers

SIR,—In your editorial "Smallpox: ignorance is never bliss" (11 January, page 75), you advocate a new breed of medically qualified safety officers, universally respected. Whilst I agree that a properly professional approach to laboratory safety is required, I suggest it would be inappropriate to require medical qualifications for safety officers.

Your suggestion that "a safety officer should always be a doctor" is ill considered. The example upon which you seem to have based the proposal is misleading. Dr Mark Darlow was in a unique position at MRE Porton. He was successful because he was the right person for that particular job.

There has been a predictable reaction in the press to the whole sad saga of Birmingham. We must use the impetus of public concern to improve our scientific safety position. It is undeniable that our scientific safety has left much to be desired and that many of its practitioners have been ineffectual. We should introduce a system for recognition of professional safety officers combined with a worthwhile career structure to encourage people in whom both the general public and the scientific community can feel confident.

The status of safety officers should be assured by their employers—who are ultimately responsible for safety under the Health and Safety at Work Act (1974). Safety officers are charged with obligations to ensure the observance of statutory regulations and safety codes. They should work in conjunction with a safety committee constituted to represent all groups of employees from academic staff to ancillary staff.

Thus it will only be when properly qualified and professionally accountable safety officers are employed and given the opportunity and incentive to carry out research and to get on with the job of safety in science, that we could feel that we have learned the lessons so apparent in this latest tragedy.

Yours faithfully,

M. R. BAILEY

National Institute for Medical Research,
London, UK.

Reason not ruction

SIR,—In your issue of 21/28 December, page 753, Dr Kenneth Mellanby quotes a secular hymn from a Socialist Sunday School in Glasgow in the 1920s:

"By nature not nurture we'll rise to the skies
The means of production we'll nationalise."

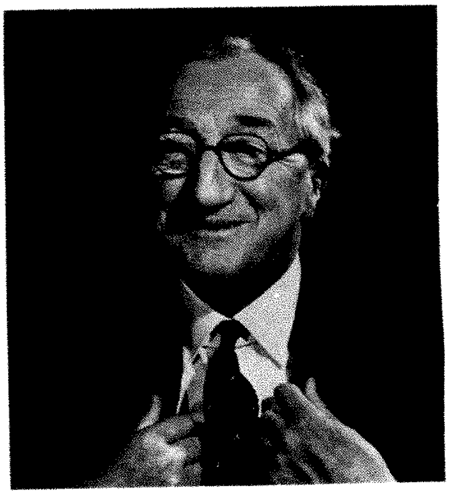
This is curiously like the refrain from a revue song by A. P. Herbert, which I quote from memory:

"By reason not ruction we'll soar to the skies
The means of production we'll nationalise
And rapture surprising we'll bring within range
By nationalising the means of exchange."

I prefer APH's version.

Yours faithfully,

O. MAYO



A. P. Herbert: British humourist

news and views

Periodical cicadas

from Robert M. May

In 1970, Princeton University conferred an honorary degree on Bob Dylan. The graduation ceremony coincided with the local emergence of periodical cicadas, which appear in vast numbers every 17 years, and the air was dense with their bodies and their song. Dylan recorded the occasion ('Day of the Locust' on *New Morning*, Columbia PC-30290):

These cicadas (not locusts; Dylan's honorary degree was in Letters not Science) are a unique biological phenomenon. They comprise three species in the genus *Magicicada*, and have the longest life cycle known for any insect. Found throughout the eastern United States, the nymphs live 6 to 24 inches below the ground, where they suck juices from the roots of forest trees. Mature nymphs emerge, assume their familiar adult form, mate, lay eggs and die within the same few weeks of every seventeenth (in the north) or every thirteenth (in the south) year. The synchrony is astonishing: although different 'broods' in different regions may be out of synchrony by several years, in any one population essentially all the animals are the same age, and the three different species are invariably synchronised whenever they coexist (as they commonly do). As far as anyone has been able to tell by conventional taxonomy or by the song of the males, each of the three species exists with both a 17-year and 13-year life cycle.

The difficult questions that are posed by the existence of these remarkable creatures are lucidly set out in the classic papers of Lloyd and Dybas (*Evolution* **20**, 133; 466; 1966) There are two basic problems. First, how did such a cycle, with so long a period, originally evolve? Second, how is the clear-cut periodicity and the synchrony between co-occurring species maintained?

The second question is more easily answered than the first. Given that there already is a long life cycle and a nearly-perfect periodicity of emergence, the cicadas can effectively escape predation. Above-ground predators such as birds, who have no analogous resource in the long intervals, are satiated, and below-ground predators, such as moles, are unexpectedly deprived of food by every periodical emergence. The resulting population densities achieved by periodical cicadas are one or two orders of magnitude greater than those of their more stodgy relatives that appear each year. Lloyd and Dybas estimate that the nymphs may typically attain densities of one ton per acre. The selective pressures are for doing well at high population densities, even at the cost of being conspicuous and sluggish ('predator-foolhardy' is Lloyd and Dybas' term). Any mutant, migrant or developmentally different individual who fails to emerge with the majority will almost certainly be snapped up by predators. Lloyd and Dybas review observations and experiments bearing this out: typically the cicadas that emerge during the first few days are all eaten by birds; in 1889 Marlatt transported large quantities of eggs to an area where the adults that emerged in 1906 were out of phase, and the entire colony of more than 10,000 individuals was effortlessly consumed by grackles In brief, the long-period cycle enables the cicadas to saturate their predators, which reciprocally helps to stabilise the cycle as individuals appearing earlier or later are gobbled up

This conspicuous association between predation and periodical behaviour has led many people to speculate that the magic numbers 13 and 17 arise because they are prime, thus preventing any possible subharmonic resonances by the predators. Robert MacArthur remarked that it may be the only application of number theory, as such, in mathematical biology I am surprised nobody has yet suggested that the prime number 11 is missing because it permits resonances with the sunspot cycle!

Two recent papers have developed some of these notions more formally, providing useful insights.

Hoppensteadt and Keller (*Science* **194**, 335; 1976) present a mathematical study whose essentials are simple. The cicadas have a life cycle of length L years. All 'broods' may be present, that is, the periodical emergence is not assumed. Each year the nymphs in the appropriate brood emerge and become adult, with each surviving adult producing an average of f offspring. Nymphs belonging to the other broods suffer some mortality, with a proportion α surviving into the next year. The newly hatched nymphs become established underground, subject to the constraint that the total number of nymphs present in the ground in any one year is limited to be not more than some carrying capacity K. Finally, the adults suffer predation, up to a saturation level that depends on the predator density if the number of cicadas is below this saturation level, all are eaten; if above, the excess survive to reproduce. The predator density depends on the previous year's density (reduced by a mortality factor R) and on the previous year's recruitment (proportional to the number of cicadas consumed that year) This dynamic model of the interaction between cicadas and their predators can have three distinct outcomes If predation is too severe, the cicadas cannot survive, and the system collapses. If predation is relatively unimportant, a 'balanced' equilibrium in which a constant number of cicadas emerge each year is possible, and seems to be the only stable state. Most interesting is the case where predation is of an intermediate strength, such that a balanced state is not possible, but a periodical state (with just one brood, emerging every L years) is possible. The criterion for this intermediate situation to hold is that

$$\left(\frac{AF}{1-F}\right) > 1-R > \left(\frac{AF}{F-1}\right)\frac{R^{L-1}(1-R)}{(1-R^{L})}$$

Here R and L are as defined above, $F=f\alpha^{L}$ is the average number of offspring produced, and A is the efficiency

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Robert M. May is Class of 1877 Professor of Zoology at Princeton University.

at which consumed cicadas are converted into predator biomass. The mechanism underlying this intermediate case is not simply that the high cicada density associated with the periodical state saturates the predators while the balanced emergences cannot (remember that in this model higher cicada densities will generate higher predator densities), but rather that the longer periods between recruitment in the periodical state make it harder for the predators to keep their density high (hence the factor R^L on the right hand side of the equation). The above criterion is a rather delicate one to satisfy, and large values of L help to do so. Hoppensteadt and Keller arbitrarily choose parameter values which produce beguiling figures in which synchronised, periodical behaviour is manifested once the life cycle L exceeds 10 years, with balanced annual emergences for L less than this.

Hoppensteadt and Keller's model is valuable in showing how periodical behaviour can emerge as a stable phenomenon, provided that predation is of some intermediate intensity. The mechanism involves an explicit interplay between resource limitation (without which the system would either collapse or run away) and predation (without which the system would settle to a balanced state). The conclusions are not sensitive to Hoppensteadt and Keller's assumption about the dynamics of the predator population. If I replace their dynamically varying expression by a fixed predator population of magnitude P, the same three cases arise, with the interesting intermediate situation pertaining if

$$\left(\frac{1-\alpha^L}{1-\alpha}\right)\left(\frac{F}{F-1}\right) > \frac{K\alpha^L}{P} > \left(\frac{F}{F-1}\right)$$

This expression has the same general character as Hoppensteadt and Keller's. In reality, the predator population is neither entirely independent of the cicadas, nor entirely dependent on them. It is therefore reassuring that the essentials of this mechanism for producing synchronised, periodical emergences are independent of the details.

Bulmer's (*Am. Nat.* **111**, 1099; 1977) study pays more attention to competition among the nymphs under the ground. Motivated by the suggestion that periodical emergences can be produced by competition that is more severe between than within year-classes, Bulmer considers a mathematical model without predators. He finds balanced solutions if competition is fairly uniform, and periodical solutions if inter-brood competition significantly exceeds intra-brood. When the effects of predation are added (assuming a constant predator population), a conclusion similar to that of Hoppensteadt and Keller is reached: low levels of predation leave the balanced solution stable, but intermediate levels destabilise the balanced solution and produce a periodical one. In his discussion, Bulmer flouts the conventional wisdom, concluding that "competition between year-classes is the main factor likely to lead to periodical behaviour". This conclusion, however, depends crucially on whether inter-brood competition is indeed a lot more severe than intra-brood (as Bulmer assumes) or not (as Hoppensteadt and Keller assume). The facts surveyed by Lloyd and Dybas seem to me to speak for the later view: the fighting behaviour displayed by nymphs in defence of their cells suggests competition is significant; but there is great variability in size among nymphs of the same age ("the range of variation within a year is far greater than the mean difference between years", Lloyd and Dybas, *op. cit.*, page 473) which makes it unlikely that inter and intra-brood competitive interaction are greatly different.

Bulmer also considers the situation when two or three species of periodical cicada occur together, and shows that satiation of predators is a most effective mechanism for synchronising the emergences. He also compares his mathematical studies with information about the natural history of some other periodical insects, namely May beetles (which over most of their range are periodical with a 3-year life cycle, lengthening to 4 or 5 years in some regions of northern Europe) and the northern oak eggar (which has periodical behaviour with a 2-year cycle). Although these insects have much shorter cycle times, and less dramatic population behaviour, than the *Magicicada* species, Bulmer's idea of using them to help shed light on the evolution and maintenance of periodical behaviour is a nice one.

The studies by Hoppensteadt and Keller and by Bulmer both assume that the periodical cicada populations are limited by the resources available to the nymphs. Lloyd and Dybas contest this point. They suggest that more nymphs could usually be sustained in their environment, and that the overall population is limited by a fungus disease, *Massospora cicadina*, which afflicts adult cicadas; the resting spores of this fungus survive for decades, and thus amount to a form of 'predator' for whom 17 years is a short time. If Lloyd and Dybas' speculation is eventually borne out, it will constitute a striking example of the magnitude of a natural population being ultimately controlled by a disease (Anderson & May *J. Anim. Ecol.* **47**, 219; 1978).

There remains the fundamental question of how the 13 or 17-year cycle first evolved. Lloyd and Dybas survey two classes of explanation.

One envisions a periodical pattern first appearing in order to facilitate predator saturation, and then gradually lengthening. The problem here is that the very same mechanisms that maintain the periodical behaviour would select against any deviant cicada that tried to add a year to its cycle time; a cycle in which predator satiation played a crucial role could conceivably evolve to be shorter, but not longer. On this basis, the periodical cicadas seem to be sitting on an 'adaptive peak' to which there is no accessible approach.

The other type of explanation assumes an initially 'balanced' cicada population, controlled by predators and parasitoids, all of whose cycle times lengthened, perhaps for developmental reasons or perhaps driven by an evolutionary race between prey and predator. Once the cycle time L attained in this way becomes long enough (in the sense specified by the equation above), the balanced configuration can abruptly become unstable and yield a periodical one. Moreover this discontinuous change in the dynamics of the system could spell extinction for some of the previously key parasitoids, thus making the situation observed today all the more enigmatic. This is pretty vague stuff, but there is at least some evidence that cycle times for insect prey and predators can be long. Other species of forest cicada for which the life cycles are known have 7-year cycles; long cycle times may well be common among cicadas with more or less 'balanced' behaviour. Among their predators, the large Australian cicada-hunting wasp, *Exeirus lateritius*, has facultative dormancy of 2 or 3 years, and some of the parasitoids of the wheat blossom midge, *Sitodiplosis mosellana* (which itself has a dormancy underground of up to 13 years), exhibit dormancy periods of up to 8 years. Recent work on models for host-parasitoid systems with mixed developmental periods for the hosts and with parasitoids whose cycles are submultiples of their hosts' (MacDonald *Math. Biosci.* **31**, 255; 1976; Beddington, Free & Lawton *Nature* **273**, 513; 1978) lay the foundations for a more quantitative exploration of these ideas.

Finally, a kind of experimental test between the above two general explanations is possible. On the first class of explanation, we would expect the 13-year cicadas to have appeared first, and the 17-year ones to have evolved from them. On the second class of explanation (which acknowledges that once periodical behaviour becomes established, cycle times can only evolve to be shorter), the 13-year cicadas would

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be derived from the 17-year ones. White and Lloyd (*Am. Midl. Nat.* **94**, 127; 1975) have shown that the 13-year cicadas have five instars (the last of 4 years' duration), while the 17-year ones have an extra, sixth instar of 4 years' duration If the second explanation is correct, the 13-year cicadas evolved by dropping an instar, and they should be replacing the 17 year ones whenever they are in contact. Such regions of contiguity exist, for example, in the Ozark mountains, and detailed studies of future emergences in this region will be interesting. This touches on a practical problem in this research, namely that, whatever their significance in the world of pedators and parasitoids, 13 and 17 years are much longer than the time scale for most research grants and tenure decisions.

To summarise, it is hard to see how periodical behaviour can evolve. In particular, the Hoppensteadt-Keller criterion can only be satisfied by a delicate juggling of the pertinent parameters. But it should be remembered that the periodical cicadas comprise only 3 of some 1,500 cicada species; an easy explanation for the phenomenon would be an embarrassment! ☐

Testing quantum chromodynamics

from Frank Close

OF the four natural forces only electromagnetism and gravity are very well understood. Theoretical developments during the past few years have produced candidate theories of the other two natural forces—the weak force of radioactivity and the strong nuclear force. 1978 saw the dramatic and extremely beautiful experiment at Stanford, California which discovered parity violation in electron-proton scattering (*News & Views* **274**, 11, 1978). This provided significant evidence supporting the model of the weak interaction developed over seventeen years by Glashow, Salam and Ward and by Weinberg and which became a candidate theory following work of 't Hooft in 1971. For the first time we appear to have a theory of the weak force which violates no sacred principles such as relativity, unitarity and causality, is renormalisable (does not have unwelcome infinite probabilities in it), is being increasingly confirmed by data—and could even be correct.

In addition to having apparently uncovered another of Nature's secrets we have obtained a bonus, namely that the weak and electromagnetic interactions are not two independent phenomena but are intimately unified (an intellectual achievement comparable with Maxwell's unification of electricity and magnetism). The above success has been brewing for the past 5 years and has been well described in the scientific press (*Nature* **264**, 505; 1976; **273**, 99; **274**, 11; **275**, 267; 1978). Meanwhile, encouraged by this achievement much enthusiasm and effort has been devoted to developing a theory of the strong force—the one remaining force without a satisfactory candidate theory.

An elegant theory of the strong interaction has been recently developed and is known as 'quantum chromodynamics'. It has all the desirable necessary theoretical properties outlined above for the weak theory, and indirect clues gathered over the past few years have provided qualitative support for it. What has been lacking is a quantitative test whereby the theory can be eliminated if it fails. Two experimental collaborations at CERN, Geneva, have performed such a test and the results appear to be in agreement with the theory. Hence 1978 may go down in history as the year in which two of Nature's forces were at last revealed.

A theory of quark interactions

Quantum chromodynamics (QCD), the theory of the strong interaction, is almost identical to quantum electrodynamics (QED), the theory of the electromagnetic interaction. There is one minor difference between them which turn out to have far reaching consequences.

In QED the fundamental particles (leptons and quarks) carry electrical charge and couple to photons, the quanta of the electromagnetic field. The strength of the coupling is proportional to the charge of the particle There is only one type of charge; like real numbers it can be positive or negative Mathematically this is a U(1) field theory. Particles which do not contain electrical charge do not couple to the photon Hence electrically neutral neutrinos do not couple to photons, nor do photons couple to other photons directly as they are also electrically neutral

It has been known for several years that quarks carry another type of charge called colour. Leptons (such as the electron) do not possess this charge. One immediately suspects that the strong force is somehow connected with this colour charge seeing that quarks do feel the strong force and also carry colour charge whereas the colour neutral leptons do not experience the strong interaction One therefore creates a quantised field theory of chromodynamics—the interaction of coloured charge with 'gluons', the quanta of the chromodynamic field—and hopes that this will have relevance to the strong interaction This is in complete analogy to electromagnetism The gluons are the analogues of the photon and are massless, vector (spin$^+$) particles.

The one essential difference between QED and QCD is that whereas there is but one type of electrical charge in QED, the colour charge has three independent varieties (often called red, yellow and blue charges by analogy with the primary colours) Mathematically this threefold charge yields an SU(3) field theory as against U(1) for QED This has the consequence that gluons themselves carry colour charge whereas in QED photons do not carry (electrical) charge; for example a red quark annihilating with a yellow antiquark will yield an orange (red-yellow) gluon There is a total of eight coloured gluons as against just one photon in QED

Coloured gluons couple to any particle which carries colour charge. The gluons themselves have colour. Hence gluons can couple directly to other gluons. This essential difference from QED, where photons being electrically neutral do not self couple, yields profound consequences but for which our Universe would have been very different, or even nonexistent

Asymptotic freedom

QCD makes specific predictions as to the strength of the quark to gluon coupling. Its absolute magnitude is not understood (just as no-one has yet understood why the electromagnetic coupling has the magnitude $\alpha=1/137$). However, the coupling is predicted to have an effective dependence upon the interquark distance. This dependence has been confirmed in recent experiments. To illustrate what we mean, first recall the more familiar case of electromagnetism

When a charge is placed in a dielectric it polarises the surrounding material. The effective strength of this charge, as measured by the force on

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a second test charge, will depend upon the separation of the two charges because when the separation is less than the intermolecular spacing the full effect of the charges will be felt without any dilution from the polarisation of the surrounding dielectric whereas at larger separations the polarisation dilutes the charge density. If we insist that the force is Coulombic, then we will be forced into concluding that the effective charge has increased at short distances.

The same phenomenon obtains in a vacuum where an electron surrounds itself with a cloud of virtual electron-positron pairs. A detailed study of QED confirms this heuristic picture: the value of α is not a constant but increases at short distances.

In QCD the prediction is precisely the opposite: the effective coupling decreases at short distances. This is essentially because the gluons now carry charge themselves (in contrast to the photons of QED) and tend to transport the charge of the source away from the source and into the surrounding cloud of virtual quark-antiquarks and gluons.

Hence if a very high frequency short wavelength beam probes the space-time structure of the proton at short distances, it will see a collection of effectively free quarks. This phenomenon was discovered ten years ago and was somewhat paradoxical since if quarks were indeed free then why did they not float freely out from the proton?

QCD appears to provide the answer to this paradox. It predicts that the quarks are free at asymptotically short distances, as observed As the quarks are pulled apart so the force increases. Hence at large distances the interquark force is large and they are confined in the proton. This property of the quarks has suggested the comparison of the proton to an ideal prison—perfect freedom inside but no-one can escape.

Testing QCD

The above is a pleasing resolution of a paradox—free confined quarks—but is hardly sufficient to confirm a theory. What are needed are quantitative predictions to test. Last summer a collaboration from Aachen, Bonn, CERN, London, Oxford and Saclay produced data on very high energy neutrino-nucleon interactions at the CERN-SPS which appear to provide the first dramatic quantitative test of the theory (Bosetti *et al. Nucl. Phys.* **B142**, 1; 1978). A recent paper from a CERN-Dortmund-Heidelberg-Saclay collaboration (de Groot *et al.* CERN report) has provided independent confirmation of these results.

What has been shown is that there are gluons as well as quarks in the proton. The gluons have been shown indeed to be vector particles (like the photon and as required by QCD). The short distance interquark force cannot be probed to infinitesimal distances because the experiment is limited to finite energy. By tuning the energy one can measure the distance dependence of the quark-gluon coupling. The quantitative behaviour was found to be as predicted by the theory.

These are all solid clear cut tests of the theory. There are also some hints in the data that may provide support for the prediction that the gluons can couple to each other (as a result of their carrying colour charge) but this will require much more study before being fully proved.

Another test of the theory involves electron-positron annihilation. At high energies the particles produced appear to come in two jets, as expected if they are the products of a fast quark and antiquark that have been produced in the e^-e^+ annihilation. If the e^-e^+ produce a massive vector meson like the ψ/J at 3.1 GeV or the Υ at 9.4 GeV, then QCD predicts three jets. This is because the meson turns into three gluons; three being the minimum number allowed by the various conservation laws. The ψ/J is too light to allow a good test of this prediction Studies of the more massive Υ at DORIS, Hamburg, are beginning to provide data supporting the three jet prediction. These tests will be high on the list of priorities of the early physics at PETRA, the new high energy electron-positron machine at Hamburg.

In 1979 we can hope to see QCD established as the theory of the quark force. If, as seems probable, it is confirmed then we will have theories of weak, electromagnetic and strong forces. The former pair are already known to be intimately linked and unified. Their similarity in structure to QCD, if verified, hints at more than coincidence and promises that all three may be related or unified.

Ideas on this are already being developed in anticipation. Experimental confirmation of QCD will be a milestone as another of Nature's forces will have been revealed. It seems possible that the dream of synthesising all of the natural forces may soon be realised with potentially profound consequences

There is great optimism that the experimental discoveries of 1979 will be pointing us down this road. This would be an apt celebration of Einstein's centenary as it was his genius that gave us the theory of the remaining force, gravity. Furthermore he spent a substantial part of his later life attempting to build a unified theory of the natural forces. If we are indeed on the verge of verifying QCD, and if unification with the electro-weak interaction is next, then the final step will be to include gravitational interaction ☐

Immunology of malaria

from F. E. G. Cox

THE possibility of producing a vaccine against malaria, probably the most important disease in the world, is one of the major challenges facing biomedical scientists today. This is a field for optimists for, as the pessimists point out, there is no immediate possibility of such a vaccine On the other hand, over the past few years progress in our understanding of the immunology of malaria has been phenomenal and it is important at this point to take stock and to assess the possibilities of vaccination and also, assuming that an effective vaccine can be produced, the problems inherent in making it acceptable. The World Health Organisation has encouraged most of this recent work and the purpose of a recent workshop* was to review our present state of knowledge of this subject.

In malaria, sporozoites injected by a mosquito circulate in the blood for about 30 min before entering the liver to undergo the first stage of multiplication. Invasive stages, the merozoites, flood from the liver and invade red blood cells in which further multiplication occurs, resulting in more merozoites, and eventually sexual stages, gametocytes, form and these are taken up by the mosquito in which the life cycle is completed. Immunological responses to all stages of the life cycle have been demonstrated but a major problem is that these responses are stage-specific. At present, there are three main candidates for the preparation of conventional vaccines; sporozoites, merozoites and gametocytes. The sporozoite is an obvious target and the possibility of an anti-sporozoite vaccine, pioneered by R. S. Nussenzweig (New York), has been adopted by the US Navy. Successes have been reported using sporozoites inactivated

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*A Workshop on the Immunology of Malaria was held at the National Naval Medical Center, Bethesda, Maryland on 2–5 October, 1978. It was sponsored by the Naval Medical Research Institute, USAID and the World Health Organisation.

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in various ways in several host-parasite models. The main advantage of the sporozoite vaccine is that it does not need an adjuvant while the disadvantage is that it requires a number of immunising injections.

Merozoite vaccines (developed by S Cohen, London) are also effective and the material necessary for preparing the vaccine is now easily and safely obtained but adjuvants are required. Gametocyte vaccines (R W Gwadz, National Institutes of Health, Bethesda) prevent fertilisation within the mosquito but depend on the vertebrate host experiencing an infection This kind of vaccine is effective and academically interesting but hardly acceptable in its present form In addition to these conventional specific vaccines there is also evidence that nonspecific factors play a part in immunity against malaria and may involve the natural killer (NK) cells now being implicated in tumour immunology (A C. Allison, Clinical Research Centre, Harrow; H Wigzell, University of Uppsala)

It is obvious that the vaccines in use at present need to be improved and considerable effort has gone into exploiting the possession of stage-specific antigenic differences and it is in this area that biochemists are coming into their own. The antigens of *Plasmodium knowlesi* from rhesus monkeys have been well studied and merozoite antigens have been analysed by J A. Deans (London), sporozoite antigens by E Nardin (New York) and blood stage antigens by R Schmidt-Ullrich (Boston) However, this work has proceeded little further than the preparative stage at present and work with *P. falciparum* is no farther advanced (R J M. Wilson, London)

The final part of the meeting was devoted to the practicability of developing a vaccine suitable for use in humans The need for carefully controlled trials and the difficulties inherent in these were stressed by I A McGregor (Gambia) and the possibility of having to accept some risks and only partial success was raised by R D Powell (University of Chicago, Chicago). Because of the need for safety and the difficulties in finding volunteers and naive populations in endemic areas, the successful development and perfection of a vaccine may present even greater problems than discovering one.

It may be that asking a group of malaria immunologists if they believe in a vaccine is like asking the College of Cardinals if they believe in God but the general consensus of opinion from the meeting was that scientists should be asking when a malaria vaccine could be developed, not if, and this opinion must justify the World Health Organization's faith in such a possibility □

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The surface photoelectric effect

from John Pendry

One of the theoretician's favourite models of a solid is a box in which the potential is constant so that the electrons move around freely The mathematical simplicity gained by neglecting atomic scattering enables a comprehensive treatment of the problem to be made; in particular the surface of the box can be studied in detail. There is a competition between the electrons' desire to stay inside the attractive potential of the box, and the tendency to spill over the surface and so reduce the kinetic energy The situation is made doubly complicated because of the charge on the electron which means that as the electron wavefunctions are modified, the potential changes as well. A self-consistent determination of the electronic wavefunctions and the surface potential must be made to discover the height of the potential barrier at the surface, and its shape, particularly its width, which reflects the degree to which the electrons spill over the surface This surface potential is important for certain applications such as the study of chemisorption at a surface, or electrical properties of surfaces An alternative way of viewing the electrons' response to the surface is to treat them collectively as a dielectric medium which screens the bare surface potential of the original box without the electrons This collective view is particularly appropriate when a time-dependent perturbation, such as a photon, disturbs the system The electrons are caused to oscillate collectively and are known to exhibit characteristic resonances at the surface-plasma frequency. This frequency is determined by the details of the surface, as is the distribution of the charge that takes part in the collective oscillation Again these surface plasma oscillations dictate some of the basic properties of the surface

Surprisingly enough, it is believed that this idealised model of free electrons is quite close to the truth in simple metals such as sodium, potassium, magnesium or aluminium. It fails of course when the atomic character of the electron orbitals persists in the solid, as in the transition metals. The existence of a large body of theory for this tractable model, and the physical realisation of the model in several easily available materials could be expected to lead to a rapid growth in our understanding of the surface potential and the surface plasma oscillations This has not been so. Interpretation of the major experimental technique, photoemission, has many complications and it has not been easy to vary enough parameters to get a comprehensive picture Recently Petersen and coworkers (*Zeitschrift fur Physik* **B31**, 171; 1978 and *Phys Rev Lett* **41**, 1314; 1978) have used a synchrotron to provide a source of polarised photons whose energy can be varied at will in the 0-25 eV range It seems that experiments with this source can confirm many of the theoretical predictions made in recent years

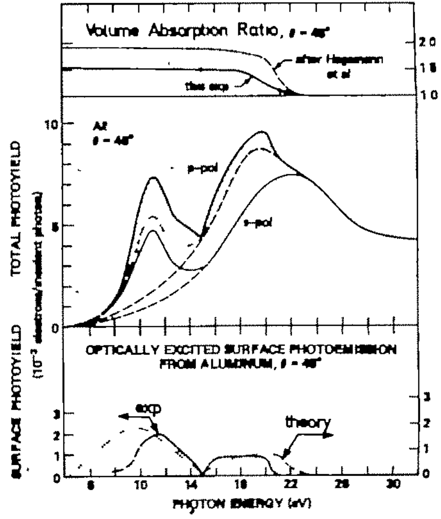

Fig. 1 The results of Petersen for s and p polarised photoemission from an aluminium surface.

In an extremely simplified picture of photoemission, the emitted current I of electrons is proportional to

$$I \propto \sum_{i} |\langle\psi_i| \mathbf{A}\cdot\nabla V |\psi_f\rangle|^2$$

where ψ_i is the electron wavefunction before excitation, ψ_f the wavefunction after excitation, **A** is the A-vector of the electromagnetic field, and V is the step in potential at the wall of the box, which confines the conduction electrons inside the box It is commonly called the surface barrier, and in the free electron model at least is a function only of the coordinate normal to the surface We can easily understand the occurrence of both **A** and ∇V in the expression for the current: the photon described by **A** provides the energy to excite the electron, but

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carries hardly any momentum, ∇V on the other hand is a source of momentum normal to the surface and allows both energy and momentum to be properly conserved in the transition. I should add that in a rigorous treatment other terms appear that are due to inhomogeneity of A(r) at the surface. Obviously our equation provides a means of checking both the variation of charge density at the surface (which determines V) and the dielectric properties (which determine A, given the conditions of incidence).

Complications arise on two accounts: (1) The potential inside the solid is not perfectly constant and ∇V will have volume in addition to surface contributions; (2) the critical parameter in the equation above is the angle between A and the surface normal. Any roughness of the surface will spoil the simple predictions Complication (2) is much more critical than might have been suspected. even small inhomogeneities in the surface can excite the surface plasma oscillations which propagate away from the inhomogeneity and change the **A** vector over a large area Both (1) and (2) have to be subtracted before comparison with theory can be made.

By changing the polarisation of the light from s ($\mathbf{A}_s$ parallel to the surface) to p ($\mathbf{A}_p$ perpendicular to $\mathbf{A}_s$) the true surface effect can be switched on. Figure 1 shows Petersen's results for s and p polarised light. When the s results have been renormalised to correct for the larger reflectivity of s light they give the contribution only for effects (1) and (2) to the p polarised results. The remainder results from the $\mathbf{A}.\nabla V$ terms which is of course zero for s light. The lower panel in Figure 1 shows comparison of the corrected experiments with theory (Endriz *Phys. Rev.* **B7**, 3464; 1973) taking account of surface plasmon effects in addition to the simple term shown in the equation. The results are encouraging but reveal that theory has some way to go before a complete description is available. At least the new generation of experiments are now providing the checks on theory required to stimulate further advances. ☐

Non-ideal plate tectonics

from Ron Oxburgh

THE general character of the large scale tectonic plates that make up the surface of the Earth is by now fairly well known So too are their present day motions as they separate, allowing warm ductile rock from below to well up and congeal onto their trailing edges; as they slide past each other, or as they converge, on sliding beneath another to be assimilated once more into the Earth's mantle Three lines of evidence may be used to constrain these motions: spreading rates, earthquake slip vectors, and the trends of transform faults, all are employed in a recent analysis of plate motions by Minster and Jordan (*J. geophys. Res.* **83**, 5331; 1978).

They estimate spreading rates from the distance of a characteristic magnetic signature on the ocean floor (anomalies 2 and $2'$) from the ridge crest at which it was formed; the rates are thus averaged over a narrow and well constrained time interval of about 3 million years Earthquake slip vectors and transform fault trends allow the direction of relative motion between two plates to be determined; the former are established by studies of the first motions associated with a particular earthquake observed in different parts of the Earth, and constrain or define the relative motion on the plate boundary where the earthquake occurs. However, the best directional constraint is provided by transform faults; they must lie along small circles parallel to the direction of movement between the two plates, as along them plates neither separate nor converge.

Minster and Jordan use 110 estimates of sea-floor spreading velocity, 78 transform fault azimuths and 142 earthquake slip vectors to construct a world-wide, internally consistent model for motions between 11 surface plates They show that this new data set provides a model which is free of most of the inconsistencies of earlier syntheses and with one major exception, fits all observations extremely well. The exception is the boundary of the Indian plate with the Antarctic and Pacific plates where there are systematic misfits Although this could be the result of observational errors the authors point out that it could also be the result of internal shortening within the Indian plate in a northwest–southeast direction at a rate of about $1\ \text{cm yr}^{-1}$.

Clearly one must hesitate before accepting an interpretation which involves an assumption diametrically opposed to that upon which the model was constructed in the first place, that is, that plates are internally rigid One must ask whether in that case the Indian plate is the only one undergoing internal deformation and, if not, whether the fit achieved elsewhere may not be fortuitously good.

The answer to these questions is probably that the authors are right. A somewhat improbable pattern of observational errors would be required; and, on the other hand, the Indian plate is sufficiently unusual in several other respects that it could be different in this way too; not only did it move northwards during the Mesozoic at, by plate tectonic standards, the breakneck speed of $17\ \text{cm yr}^{-1}$, but after the collision of the Indian and Eurasian continents continued to do so at a significant rate (the Minster and Jordan data suggest about $5\ \text{cm yr}^{-1}$). The interaction of the two continental masses must interfere with steady subduction along the northern boundary of the plate and give rise to anomalous stress conditions within it. It is worth pointing out that the plate is also the site of a massive and unexplained geoid anomaly—the geoid (the gravitational equipotential surface) is depressed about 110 m below its expected value.

The interaction of the Indian and Eurasian plates finds its most spectacular expression in the Himalayan Mountain chain Very different in character, but apparently developed almost simultaneously, is the Tibetan plateau immediately to the north. The plateau is a lozenge-shaped flat area of over a million km^2 bounded by mountainous zones of folded and faulted rocks. The plateau was beneath sea level at the end of the Cretaceous period and has subsequently been elevated without significant folding to a height of about 5,000 m. There has been extensive young volcanic activity within it during the uplift and continuing to the present.

Relatively little is known, at any rate in the West, of the geology of this fascinating area It is therefore hardly surprising that two separate groups, Molnar and Tapponnier (*J geophys Res.* **83**, 5361; 1978) and Ni and York (*J. geophys. Res.* **83**, 5377; 1978) have taken the opportunity of studying it by means of the new ERDA LANDSAT photographs and interpreting these along with slip vector information derived from earthquakes occurring within the area. Both arrive at the interesting conclusion that although in the Himalayas along the southern margin of the plateau there is thrust faulting, within the plateau there are north–south trending normal faults with the formation of rifts, and indicating an extension in an east–west direction. There is also strike-slip faulting in a WNW–ESE trend. It seems

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therefore as if the Tibetan plateau is responding to compression from the south not by folding but by a kind of lateral extrusion. This requires a ductile behaviour in the underlying crust and upper mantle, a condition which is compatible with an observed strong attenuation of elastic waves beneath the region.

Molnar and Tapponier take the further step to suggest that the ductile lower crust and upper mantle beneath the Tibetan plateau may be regarded as the working fluid in a massive geobarometer, the elevation of the plateau reflecting the level of stress transmitted to the fluid contained by surrounding but uncoupled blocks. The authors essentially propose the decoupling of a thin and more or less passive brittle, upper crustal layer from a ductile zone beneath, which experienced relatively homogenous crustal shortening. It does seem, however, that the amount of isostatically compensated crustal shortening required to 'pump up' the Tibetan plateau to 5,000 m is very large (of the order of 100%) and this would involve a much larger strain in the overlying plateau than is observed.

Molnar and Tapponier may not have solved the problem of plateau uplifts—there are other comparable but smaller features such as the Colorado plateau—but they have refocused attention on the long standing problem of how stresses are transmitted through the lithosphere to produce localised strains. The deformation is clearly inhomogeneous and there is much to be learned about the formation and decoupling and interaction of small scale crustal and lithospheric blocks. The combined application of the methods of earthquake fault-plane solutions, surface geology, gravity, and elastic wave attenuation studies offer a promising way forward. ☐

Life at low Reynold's number

from Paul Colinvaux

SMALL planktonic animals must feed in water which has properties a little like those we know in treacle, as the size and slow motions of the zooplankters make them live at low Reynold's number, at which liquid flow becomes laminar. One of the properties of laminar flow is that raking or stirring movements do not jerk particles through the water so that there is no easy displacement of suspended matter. Planktonic herbivores respond to this reality by generating a flow of water towards themselves and then 'filtering' the flow for algal food. Operating a filter seems an indiscriminate mode of feeding and one wonders whether one of the consequences of life at low Reynold's number is that zooplankters are of so catholic a taste that they eat everything caught in their 'filters'. If they do, it seems curious that the algae of the phytoplankton have evolved so many shapes and sizes. Zooplankters must also cope with the consequences of living in a transparent medium. Rapid motion is forbidden by the low Reynold's number and there is nowhere to hide. Their fluid environment also tends to be layered, warm on top and cold underneath. The strategies adopted by zooplankters to deal with these conditions were explored at a conference on the structure of zooplankton communities held at Dartmouth College New Hampshire*.

We now have a better idea of what 'filtering' really means because the process has been watched by high-speed cinematography through a microscope (J. R. Strickler, University of Ottawa; J. J. Gilbert, Dartmouth College). It turns out that food is often 'handled' rather than 'screened'. A long filamentous diatom colony, for instance, is manoeuvred by a copepod to be end-on to the mouth, then fed into the jaws and broken up like brittle spaghetti. Larger fragments vanish down the animal's mouth but smaller fragments drift away. In a feeding rotifer, a stream of water flows against the ciliated wheel, where different algal cells are accepted or rejected as they thump against the cone that leads to the rotifer mouth.

Data on what particles are removed from suspended mixtures also show that herbivores in the plankton do specialise to some extent but that the discrimination is fairly crude, usually by size or against the obviously scarcely edible. Copepods and rotifers discriminate more than do cladocerans.

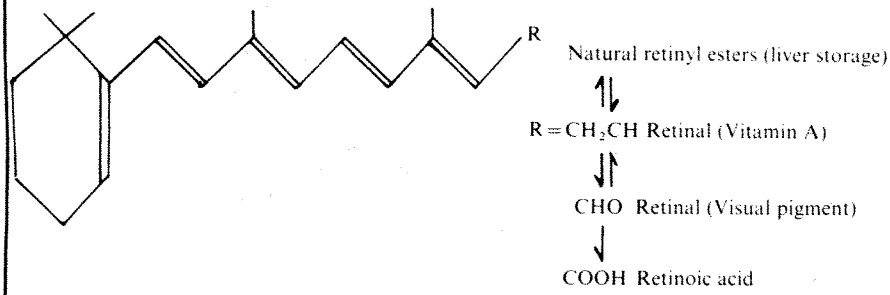
The habit of swimming up by night and down by day (vertical migration) has various possible advantages. It hides zooplankton from predators that hunt by sight. But the habit can also result in a systematic food search and it also ought to let plankters lower the energy cost of respiration by putting them in the cold bottom water when not actively feeding. The conference was given strong evidence that all three mechanisms were, indeed, driving the habit, with a suggestion that predation was the most prevalent.

Water mites, which are apparently without serious predators, may migrate up and down, apparently in quest of food or optimum temperature. But plankton in tropical Lake Gatun abandoned a former pattern of vertical migration when their fish predators were removed. In Lake Llano in the Phillipines *Chaoborus* is restricted to water deep enough to let it hide from fish at the dark bottom by day. In the 30 m high experimental chamber at Dalhousie University marine copepods went up and down when the water was essentially without food but abandoned their migrations to remain at an introduced layer of algae. In the Arctic Alaskan Toolik Lake, where there is no night in summer, there was no regular vertical migration but the animals adopted a depth which depended on temperature or their age and hunger.

Another way in which the larger zooplankton avoid predators appears to be by 'cyclomorphosis'. Specimens of some large Cladocera have a significant part of their total volume taken up by essentially transparent protuberances, a change of shape of the growing animal that can be expected to minimise the target presented to a fish

Erratum

In the article 'Epithelial cancer, differentiation and vitamin A' (*News and Views* **277**, 261; 1979) the wrong illustration was inadvertently included. The correct diagram is given below.


R
Natural retinyl esters (liver storage)
R = CH₂CH Retinal (Vitamin A)
CHO Retinal (Visual pigment)
COOH Retinoic acid

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*Held on 21-25 August, 1978.

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hunting by sight. There were more confirmatory data to support the validity of this hypothesis, particularly of the kind that correlates cyclomorphic phenomena with the presence of fish. In addition, it has been nicely shown that the visual acuity of planktiverous fish is of the order expected by the hypothesis so that reducing apparent size is a valid strategy for plankters. There is consensus that predation is, indeed, the selective force behind the cyclomorphosis of the larger cladocerans.

Attention has now shifted to the adaptive responses produced by small invertebrate predators. Some of these are at low Reynold's number like their prey, and have comparatively low success rates. Others, like *Chaoborus*, can cross the Reynold's number divide, make fast capture movements, and wait in ambush on vertical migration paths. The rotifer *Asplanchna*, an animal not much larger than the other rotifers on which it preys, is most successful against victims that are not spiny, suggesting that spines are an adaptation to *Asplanchna* predation. *Heterecope* predation can induce the possession of spines in prey populations rather as fish predation induces cyclomorphosis in Cladocera. There was a general feeling at the conference that small predators may be more important in producing structure in zooplankton communities than competition between herbivores.

With filtering, vertical migration, and cyclomorphosis becoming understood we may claim to be mastering some of the immmediate causes of community structure in the plankton. The underlying causes remain obscure. The difficulty in deciding even whether responses involve selection or merely phenotypic plasticity can be gauged by the fact that there still seems uncertainty about what constitutes many planktonic species. Taxonomists hesitate to put names on tropical zooplankters, not feeling sure whether many of these can be fairly assigned to species known from temperate latitudes, and there seems a growing doubt that species from temperate Europe and North America are really the same even though they look extremely similar. A further question is how much of the response to selective pressures such as predation can be genetic when typical populations are asexual clones?

One particular peculiarity was called to mind by several observations from the tropics: there are usually very few species of zooplankter in the open water of a tropical lake, perhaps one or two copepods, one or two cladocerans, and a few rotifers. This comparatively tiny assemblage of herbivores feeds on an array of phytoplankton species that may number 200 or so different kinds. On land, such an array of plants always seems to be under pressure from many different herbivores, and we call on the 'cropping principle' to explain plant diversity. It is an apparent paradox of the zooplankton that natural selection has not provided an array of herbivore species to specialise on the diverse array of plants. Probably the answer to this paradox lies in the fact that the discriminatory powers of planktonic herbivores, so clearly revealed at this conference, are yet rudimentary compared with those of small terrestrial herbivores. ☐

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Magnetics, climate and eccentricity

from Peter J. Smith

ONE of the most surprising correlations to have been discovered in recent years is that between fluctuations in palaeoclimate and the palaeomagnetic field—surprising because it is difficult to imagine any possible causal connection between an atmospheric phenomenon and motions in the Earth's fluid outer core. Indeed, so astonishing was the geomagnetism–climate relationship that, when its existence was first hinted at, a good many people were inclined not to believe it. But if there are still any sceptics around, it may be presumed that they never saw the report by Wollin *et al.* (*Geophys. Res. Lett.* **4,** 267; 1977) published some 18 months or so ago. For in it Wollin and his colleagues presented data from several deep sea sediment cores, showing a convincing general (anti) correlation between the intensity of core magnetisation and foraminiferal abundance—which means a correlation between warm climatic stages and periods of low geomagnetic field intensity.

They went even further. Harrison (*Earth-Sci. Rev.* **10,** 1; 1974) and Kent and Opdyke (*Eos* **57,** 237; 1976) had already suggested that any relationship between geomagnetic field and climate may be the result of a third phenomenon; and Hays *et al.* (*Science* **194,** 1121; 1976) had linked ice ages with the obliquity and precession of the Earth's orbit. Wollin and his coworkers therefore plotted not only magnetisation and foraminiferal abundance but also the variation in the Earth's orbital eccentricity as deduced by Van Woerkom (in *Climatic Change* (ed. Shapley, H.) Harvard University Press, 1953). A threefold correlation between warm climate, low field intensity and high orbital eccentricity for the past 0.8 Myr was thus revealed, the clear implication being that eccentricity modulates both climate and geomagnetic field.

In view (presumably) of previous scepticism on the climate–geomagnetism issue, Wollin *et al.* were rather cautious about the reality of the relationship emerging from their data. But if their new results are anything to go by they need not have worried, for they have now demonstrated a similar correlation in a single North Atlantic sediment core spanning no less than the whole of the past 2 million years (*Earth planet. Sci. Lett.* **41,** 395; 1978). This time they used the more recent eccentricity data from Vernekar (*Meteorol. Monograph 12*, American Meteorological Society, 1972), but the result was the same. Warm climate matches low field matches high eccentricity.

But why? For the time being that is anyone's guess; so Wollin and his colleagues have had a go at it themselves. They begin with the premise that because the density of the Earth's core is greater than that of the near-surface zone, the core–mantle boundary is less elliptical than the Earth's surface. They then argue that, as a result, the torque exerted by solar and lunar gravity on the core is smaller than that on the mantle; so the core tends to precess more slowly than the mantle. Moreover, as the Earth's orbit is eccentric, the solar gravitational field acting on the Earth has an annual variation that increases as the eccentricity increases. Therefore when the eccentricity is greater, the difference between the torques acting on mantle and core is greater, and the tendency of the core and mantle to precess at different rates is enhanced.

Wollin *et al.* then go on to suggest that such an increase in the differential procession will lead to perturbations in the core's convective flow of such a type as to reduce the strength of the geomagnetic dipole and hence reduce the effectiveness of the magnetic shield against corpuscular radiation. Anyone else want to try?

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articles

Effect of climatic warming on the West Antarctic ice sheet

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Climatic warming could cause increased melting from Antarctic ice shelves. Continued weakening of the ice shelves in this way would result in the ultimate collapse of most of the West Antarctic ice sheet. For complete removal of the ice shelves collapse of the ice sheet and a 5 m rise in world sea level could occur in <100 yr. More realistically, ice-shelf deterioration is likely to be a rather slow process, and even for a major and sustained warming trend ice-sheet collapse would take several hundred years, with most of the associated rise in sea level occurring during the final century. However, little is known about the glaciers that drain the northern part of the ice sheet. These glaciers have little or no protective fringe of ice shelf and, unless they flow over sufficiently high bedrock sills, they may show a more rapid response to increased temperatures.

MERCER warned recently[1] that increasing levels of atmospheric CO$_2$ may lead to climatic warming that is sufficiently magnified in high latitudes to cause the demise of the Antarctic ice shelves and the inevitable collapse of the West Antarctic ice sheet, leading to a 5 m rise in world sea level. Although at present, parts of the Ross Ice Shelf seem to be growing thicker[2], Mercer's warning cannot be dismissed lightly as the West Antarctic ice sheet is undoubtedly buttressed by ice shelves, and these ice shelves are highly susceptible to changes in climate. Mercer suggested[2] that significant climatic warming may be imminent if not already in progress, and here we try to estimate how rapidly ice-sheet collapse could occur and which parts of the ice sheet are most susceptible. First, however, we stress the need for more sophisticated modelling of climatic response to changing levels of atmospheric CO$_2$ since there is no general agreement regarding the sign, let alone the magnitude, of the response. Here we shall simply assume that warming, for whatever reason, is sufficient to increase ablation rates from Antarctic ice shelves.

The West Antarctic ice sheet (Fig. 1) rests on land that, for the most part, is below sea level[3]. Most of the drainage from the ice sheet is into the Ross and Ronne ice shelves, which have a stabilising influence as ice flowing across the grounding line has to push the ice shelf past its sides and around areas where the ice shelf has locally run aground on seabed shoals to form ice rises. The effect of climatic warming would be to weaken the ice shelves and reduce their buttressing effect so that flow rates from the ice sheet would increase. This would provide negative feedback because the advection of thicker ice from upstream would tend to balance the increased melting rates. In this way collapse of the West Antarctic ice sheet would proceed at a slower rate than would be the case for complete removal of the ice shelves. A full analysis of ice-sheet collapse in these conditions is extremely difficult, but we can estimate maximum retreat rates by making some simplifying assumptions.

Warmer temperatures will weaken ice shelves in two ways—by thinning due to melting at the upper and lower surfaces; and by enhancing existing lines of weakness, such as rifts and bottom crevasses, so that calving rates are increased. Disintegration of an ice shelf by such calving is most likely where the ice shelf is in longitudinal tension. This condition holds over most of an ice-shelf surface except upstream from an ice rise, where longitudinal stresses are strongly compressive and calving is unlikely to occur until the ice rise has disappeared. Once this happens the ice shelf is in longitudinal tension all the way from the grounding line to the ice front and, if accelerated calving does occur, the calving front may recede to the grounding line. In order to incorporate the effects of both increased calving and increased melting within a mathematically tractable model we shall assume that most of the Ross Ice Shelf is instantaneously removed by accelerated calving along existing lines of weakness (Fig. 1). Only the portions of ice shelf that are protected by ice rises remain and these persist until the ice shelf floats free from the seabed shoals beneath the ice rises. We shall restrict our analysis to the two most active ice streams (B and E in Fig. 1) that flow into the Ross Ice Shelf. These ice streams flow along bedrock troughs and they are separated from neighbouring ice streams by ridges or domes of slow-moving ice that rests on bedrock ridges[3]. Outflow from both ice streams is currently restricted by two ice rises within the Ross Ice Shelf—Crary Ice Rise and Roosevelt Island (Fig. 1). We assume that the ice streams are parallel sided and that, as the grounding line retreats, a floating ice shelf forms between the marginal ice ridges (Fig. 2). The seaward grounding line of these ridges will also retreat, but rather slowly because the ice is probably frozen[4] to the comparatively shallow bedrock. In our analysis we assume that during ice-sheet collapse the only restrictions to outflow are

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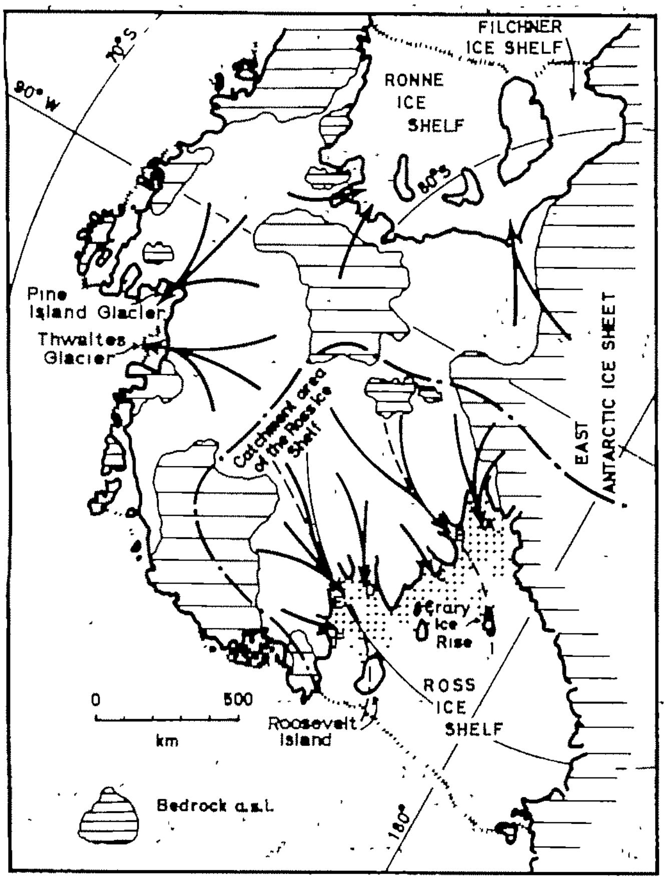

Fig. 1 West Antarctica, showing the areas of bedrock that are above sea level. The arrows represent ice streams that drain the ice sheet. Most of the ice that flows into the Ross Ice Shelf from West Antarctica drains through ice streams B and E. Pine Island Glacier and Thwaites Glacier carry most of the ice that drains northwards. These glaciers seem to be relatively unprotected by bounded or partially grounded ice shelves. Vertical sections taken along the broken lines through ice streams B and E are shown in Fig. 3. In order to calculate maximum ice-sheet retreat rates resulting from climatic warming we assume that most of the Ross Ice Shelf disintegrates very rapidly by calving of icebergs, to leave only the stippled area upstream of the ice rises.

the two ice rises and shear at the sides of the ice shelf that forms within the channel to seaward of the grounding line (Fig. 2). By neglecting the restraining effects of the rest of the Ross Ice Shelf we allow the possibility that accelerated calving may occur, and by assuming instantaneous removal of this portion of the ice shelf we ensure that calculated retreat rates represent reasonable upper limits. We admit that even higher retreat rates can be obtained if we assume instantaneous removal of the entire ice shelf. We have calculated these and we include them in our results, but we stress that they represent limiting values which could never be achieved.

Increased melting will weaken the coupling between the ice rises and the ice shelf, but ice rises will continue to exist and to limit ice-stream flow rates until the ice shelf becomes too thin to run aground. By modelling the form of the ice shelf[5] for given grounding-line positions, marginal constraints, ablation rates and values of the compressive force F between ice shelf and ice rise, we can find the minimum velocity V across the grounding line necessary for the ice shelf to remain grounded on a given shoal. Thus, there is a relationship between F and $V[V=f(F)]$ which holds while the ice rise exists. In addition, equilibrium conditions at the grounding line give an independent relationship[6] between F and $V[V=g(F)]$ in terms of snow accumulation over the ice sheet and of grounding line retreat rate. If the solution of these two equations gives positive values of F and V for a given position of the grounding line then the ice rise continues to exist and the grounding-line retreat rate can be calculated from V. As $F\rightarrow 0$ the influence of the ice rise diminishes until it becomes ungrounded at $F=0$. Thereafter we consider two possibilities: instantaneous calving of the remaining ice shelf so that further retreat is unrestricted, or persistence of ice shelf within the ice-stream channel so that retreat is delayed by shear at the ice-shelf sides. Retreat of the grounding line between ice sheet and ice shelf occurs because the thinning effects of creep and ablation are greater than thickening by snow accumulation and the advection of thicker ice from upstream[6]. In order to calculate the rate of retreat we have used available measurements of snowfall over West Antarctica[7], ice-shelf flow parameters from observations on existing ice shelves[8], and a value for shear stress between the ice shelf and its sides (0.5 bar) which is consistent with observations of floating ice that is flowing rapidly between parallel sides[9]. The effects of advection are incorporated by adopting a relationship between ice-stream velocity V and the product of ice thickness H and surface slope α: $V=C(H\alpha)^m$, where $m=3$, and $C(\sim 550\ \mathrm{m}^{-2}\ \mathrm{yr}^{-1})$ is calculated from the present-day values of $V\sim 550\ \mathrm{m\ yr}^{-1}$ (ref. 10) and $H\alpha\sim 1$ m (ref. 3 and Fig. 3) from near the grounding line of ice stream B. This form of glacier sliding law has some support from theory[11], but it is not expected to be generally valid. Here it is used solely to calculate values of α at high ice velocities, and we have chosen the highest probable values of m and C so that we obtain the lowest probable values of surface slope. This means that we probably underestimate the ice advection term and overestimate grounding-line retreat rates. This approximation is clearly a weakness in our model but it cannot be improved until more is known about the dynamics of fast glaciers and ice streams. Meanwhile our approach is useful as it gives an estimate of the probable minimum time for ice-sheet collapse.

Creep thinning at the grounding line increases rapidly with ice thickness[12], and decreases with increasing back pressure due to ice rises and shear between the ice shelf and its sides[13]. The longitudinal profiles[3] of ice streams B and E are shown in Fig. 3. Once the grounding line retreats down the bottom slope into the deep basin beneath ice stream B creep thinning is controlled by the large values of ice thickness, and retreat is very rapid even for the model that allows increasing back pressure to be exerted by the ice shelf that is assumed to form seaward. Figure 3 shows grounding-line position during retreat plotted against time for different rates of melting beneath the ice shelf. In contrast ice stream E has a comparatively horizontal bed so that creep thinning decreases as the grounding line retreats to leave an ever larger ice shelf within the ice-stream channel. In these condi-

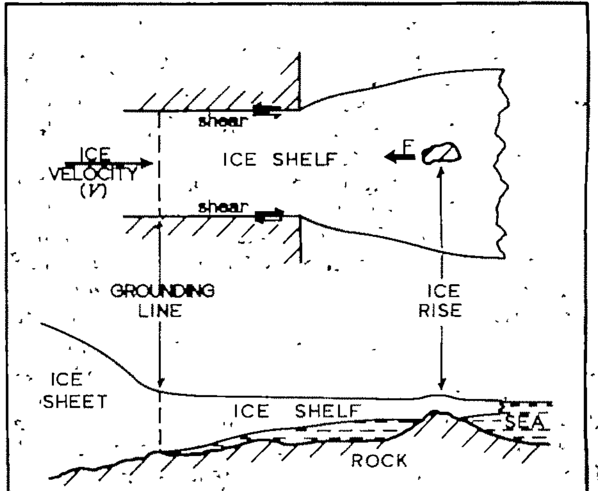

Fig. 2 A retreating ice stream. As the grounding line retreats ice shelf forms between the ridges of slow moving ice that formed the margins of the ice stream. Movement of the ice shelf is restricted by shear past its sides and by the presence of an ice rise where the ice shelf locally has run aground.

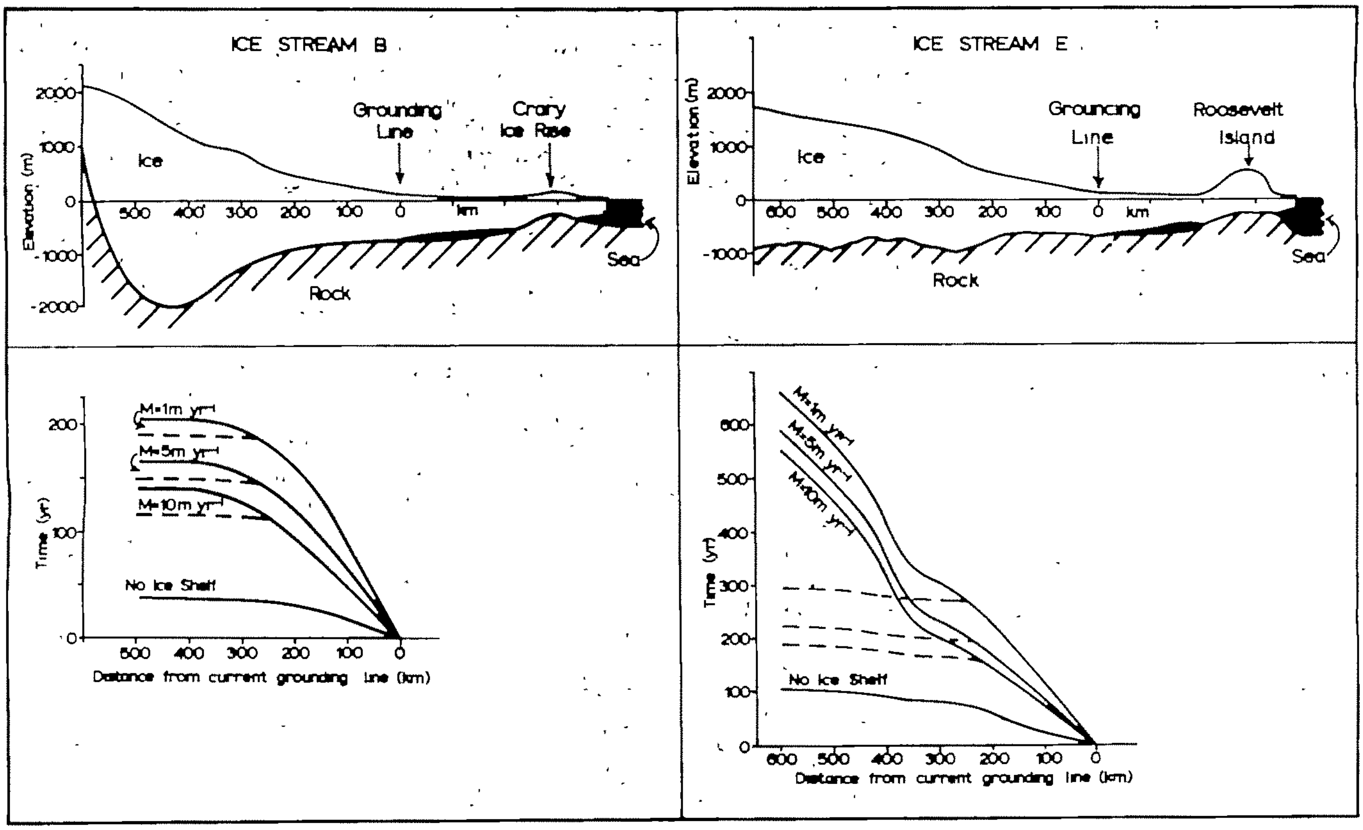

Fig. 3 The longitudinal profiles of ice streams B and E as they are today, and plots of grounding-line position versus time for these ice streams during retreat. Retreat is most rapid if the ice shelf is completely removed, and least rapid for low values of ice-shelf melt rate, *M*. Once the grounding line reaches the deep basin beneath ice stream B retreat is extremely rapid. In contrast, ice stream E flows over a comparatively horizontal bed and retreat rates are ultimately determined by the rate of collapse of the ice-shelf margins. The broken lines show retreat following complete breakup of the ice shelf as the ice rises collapse

tions the grounding line may achieve a quasi-equilibrium position once the ice shelf is so large that creep thinning is sufficiently reduced. However, the ice-shelf margins are themselves retreating and the ice-stream grounding line retreats at approximately the same rate (Fig. 3). Changing the melt rates affects only the time taken for the ice rises to collapse. This is because we have not altered the ablation rate at the grounding line, which we have assumed to be 1 m yr^{-1} in all cases. Unless the climate changes dramatically this is a probable maximum for the ice streams. Melting rates beneath ice shelves may be very high[14,15] and our model allows values up to 10 m yr^{-1} at the ice rise with a linear variation between grounding line and ice rise. Our results underestimate the influence of the ice rises because we have allowed unrestricted lateral creep once the floating ice leaves the ice-stream channels. In reality, neighbouring areas of ice shelf would restrict lateral creep so that vertical thinning would be reduced and the ice rises would survive longer than calculated. Thus, once again, our model overestimates probable retreat rates.

Figure 3 includes plots of grounding-line position against time for retreat with icebergs calving at the grounding line so that there is no ice shelf to restrict outflow. In these conditions collapse of ice streams B and E could occur within 40 and 110 yr respectively, and the rapidly retreating ice stream B would probably capture portions of the catchment areas of neighbouring ice streams to render them more and more unstable. In this way, without the protection of the Ross Ice Shelf, most of the West Antarctic ice sheet could almost completely collapse within 100 yr. However, if we allow ice shelves to persist upstream of the ice rises, retreat is significantly slowed so that total collapse of ice stream B takes between 100 and 200 yr depending on whether ice shelf survives after the ice rises collapse. Equivalent values for ice stream E are 200 and 700 yr but, because of feedback effects, retreat would be accelerated to correspond more closely to that of ice stream B, so that most of the West Antarctic ice sheet could collapse within ~200 yr. However, our assumptions throughout have been biassed to give maximum retreat rates and we stress that we have neglected the buttressing effect of most of the present-day Ross Ice Shelf. Currently, the grounding line of ice stream B seems to be advancing into the ice shelf[2], and in the recent past there may have been widespread grounding and subsequent thickening in the lower reaches of ice stream C (ref. 4). Thus, our estimated collapse rates would apply only after removal of large areas of ice shelf following a major increase in melt rates, probably from the ice/water interface. The shift in oceanographic parameters necessary to effect such an increase would be a rather slow response to climatic change[16], and our calculations take no account of how long this would take. We expect the response time of the oceans to be of the order of hundreds of years[17], so that initial retreat would be far slower than our calculations suggest. Later, as the ocean feels the effect of changing climate and as more of the ice shelf disintegrates, our model would become progressively more applicable. In effect this means that, after the climate starts to change, the first 100–200 km of retreat would take some hundreds of years and then the retreat would follow the pattern shown in Fig. 3. The entire sequence would probably take at least 400 yr, even for a major warming trend of 5 °C per century, and most of the collapse and its associated rise in sea level would occur during the final hundred years. An example of such a retreat sequence was the disappearance of the marine-based portion of the Laurentide Ice Sheet from Hudson Bay. The final collapse took ~200 yr[18], but this was probably the culmination of a retreat sequence that had started considerably earlier.

It is important to consider how relevant these conclusions are to the behaviour of other large ice streams in Antarctica. Available data suggest that the grounding line between West

Antarctica and Ronne Ice Shelf is well protected by large ice rises (Fig. 1) so that probable retreat rates are covered by our analysis. However, the major drainage from the north coast of the West Antarctic ice sheet is through the Thwaites and Pine Island glaciers (Fig. 1), and these terminate in small ice shelves which may or may not be buttressed by ice rises. The stability of these ice streams is dependent on suitably high rock sills near the ice-stream grounding lines. The critical depth (*d*) below sea level of these sills depends to a large extent on ice-shelf dimensions and on the presence or absence of ice rises, but if the fringing ice shelves are unrestricted then *d* may be as low as 400 m for each of these glaciers. Pine Island Glacier may flow into a bounded ice shelf about 50 km square and even this comparatively small ice shelf would exert sufficient back pressure on ice flowing across the grounding line to increase *d* from 400 m to more than 500 m. An increase in ablation rates would be particularly damaging in this area since there is little or no ice-shelf fringe to regulate drainage rates. Little is known about bedrock topography beneath these glaciers but available data suggest that they flow through deep troughs similar to that of ice stream B. If sufficiently high sills do not exist to provide stable grounding-line positions for these glaciers then the northern part of the West Antarctic ice sheet could already be collapsing. Our calculations suggest that, if the seabed at the grounding line of either glacier is 200 m deeper than the equilibrium value, then the grounding line is retreating at 1.5 km yr^{-1} with associated ice velocities of 10 km yr^{-1}. This would be sufficient to raise sea level by approximately 1 mm yr^{-1}. Preliminary estimates of Thwaites Glacier velocities greater than 2 km yr^{-1} have been made by R. Alan of the U.S. Geological Survey from a comparison of satellite imagery with aerial photographs. This is approximately the velocity appropriate to equilibrium with a sill at the critical depth *d*. If this is the case then Thwaites Glacier would be particularly susceptible to any increase in ablation rates.

We conclude that, if the CO_2 greenhouse effect does cause climatic warming that is magnified in polar regions, we do not expect a major increase in ice-shelf ablation rates until ocean warming is sufficient to increase melt rates beneath the ice. Even with a major climatic change and high ablation rates collapse of the West Antarctic ice sheet through ice streams that drain into the Ross and Ronne ice shelves would be restricted initially because the ice shelves would continue to regulate drainage rates, and total retreat would probably take more than 400 yr with most of the associated rise in sea level occurring during the final century. However, much of the ice-sheet drainage is to the north through glaciers that may be unprotected by ice shelves. Unless these glaciers flow over sufficiently high bedrock sills they may be in a very precarious state of equilibrium or they may already be collapsing, and significant warming could rapidly accelerate their collapse. In order to improve our assessment of the stability of these glaciers a detailed survey of their basal topography and velocity measurements on their floating tongues are urgently needed.

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Observations from the IDA network of attenuation and splitting during a recent earthquake

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The project for the International Deployment of Accelerometers (IDA) is intended to provide very low frequency seismic data for the study of Earth structure and source mechanisms. This article outlines preliminary results on attenuation and splitting derived from IDA recordings of a recent large earthquake.

ON 19 August 1977 a very large earthquake occurred about 200 km south west of the island of Sumbawa, Indonesia. The USNEIS hypocentral coordinates are 11.13° S, 118.40° E and 20 km depth. The origin time was 06:08:52.6 GMT. A body-wave magnitude of 7.75 has been assigned by the U.S.G.S. Stewart[1] has estimated the moment to be 10^{29} dyn cm (10^{22} Nm). As this seems to have been the most energetic earthquake since the 1965 Rat Island event it is ideal for the study of very long period free oscillations. The data we have used for this purpose are from the project for the International Deployment of Accelerometers (IDA)[2]. The six stations of the IDA network that produced good records for this event were located at Fairbanks, Alaska (CMO); Ñaña, Peru (NNA); Sutherland, Republic of South Africa (SUR); Halifax, Nova Scotia, Canada (HAL); Brasília, Brazil (BDF); and Rarotonga, Cook Islands (RAR). For the analysis presented here record lengths up to 526 h were used. Part of a typical record (RAR) is shown in Fig. 1. The first 4 h of the recording have been discarded since the large signals saturated the system. Before computing the spectrum, the larger bodily tides have been removed by a least squares fitting procedure and aftershocks have been excised and replaced by linear interpolation.

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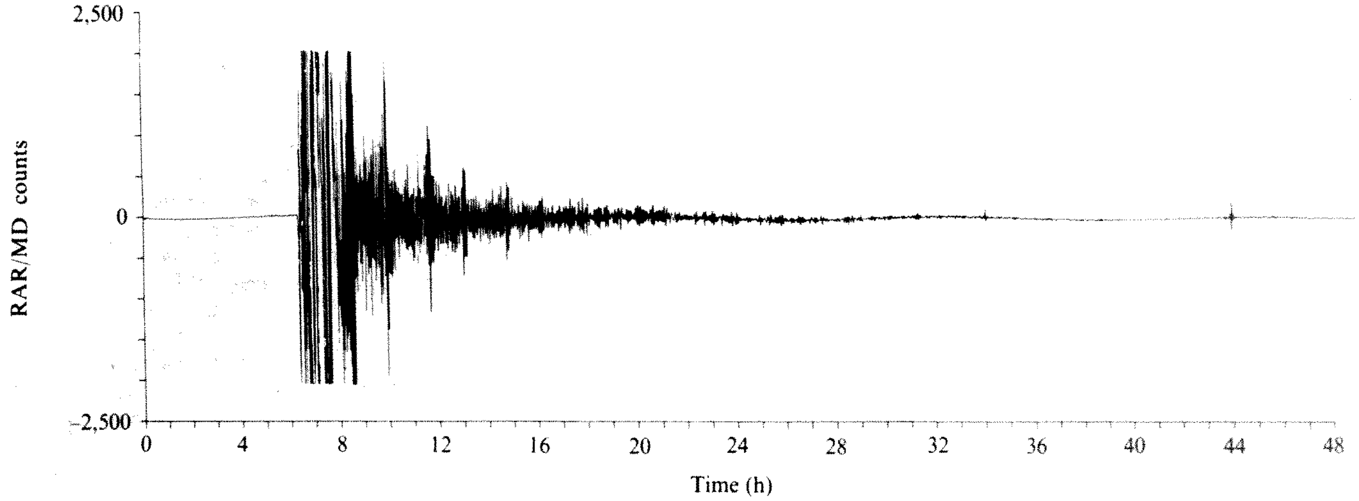

Fig. 1 A 48-h record from IDA station RAR, typical of the data from the Sumbawa event. Note that the instrument was saturated during the first 4 h of the event (through R3). Also Raleigh packets are clearly visible out to R8.

Figure 2 shows the Fourier power spectrum of 155 h of the BDF record beginning 25 h after the event. Clearly present in the spectrum are the fundamental spheroidal multiplets ${}_0S_2$–${}_0S_{21}$, several overtones, and the radial modes ${}_0S_0$ and ${}_1S_0$. The low order multiplets ${}_0S_2$–${}_0S_4$ are obviously split. The quality of the data encouraged us to examine them in more detail and we present here preliminary measurements of frequency splitting, attenuation, and degenerate multiplet frequencies.

Splitting

A multiplet ${}_nS_l$ has $2l+1$ singlet lines. Spherical symmetry causes these lines to have the same degenerate frequency. Deviations from spherical symmetry such as rotation, ellipticity, and lateral variations remove the degeneracy, wholly or partially, and cause the multiplet to be split[3] as may be clearly seen in Fig. 2 for the modes ${}_0S_2$, ${}_0S_3$, and ${}_0S_4$. To investigate this splitting, we can interpolate the discrete Fourier transform (DFT) to a frequency spacing finer than

$$\Delta f = 2\pi/T$$

where T is the length of the time series, by numerically evaluating the trigonometric series approximation to the Fourier integral. A more complete discussion is given elsewhere[4].

Figure 3 shows spectra for ${}_0S_3$ from six individual records. If we count the individual peaks in Fig. 3 we find more than the seven we know must be there. A simple calculation shows that the width (half width at half power) of each singlet is about 9×10^{-6} rad s^{-1} (due both to attenuation and finite record length). Theoretically, the average line spacing for this multiplet should be 1.36×10^{-5} rad s^{-1} (ref. 5). Clearly, the problem is interference between the strong and weak singlets.

There are three ways we know of to resolve the singlets. The first is to increase the record length. Doubling the time series length would narrow the lines to 5.5×10^{-6} rad s^{-1}, but the low amplitude singlets would be overwhelmed by noise. The second method is analytical continuation[4]. This would narrow the lines to 5×10^{-6} rad s^{-1}, but would also decrease the signal-to-noise ratio (S/N) unacceptably. The third method is stacking[6], which enhances the S/N and tends to cancel adjacent singlets. Thus, the only practicable method requires the use of the stations as an array.

At frequencies below 1 mHz we expect that the dominant perturbation from spherical symmetry will be the Coriolis force. Thus, to zeroth order in perturbation theory, the radial (vertical) component of motion of a singlet is proportional to $Y_l^m(\theta, \psi)$, the complex spherical harmonic, in geographical (north polar) coordinates. Each singlet, except $m=0$, is a travelling wave[7]. Let $U(\omega, \theta_j, \psi_j)$ be the spectrum of the vertical component at the j^{th} station. For an isolated multiplet ${}_nS_l$

$$U(\omega, \theta_j, \psi_j) = \sum_{m=-l}^{l} {}_nA_l^m Y_l^m(\theta_j, \psi_j)\, {}_nC_l^m(\omega)$$

where ${}_nA_l^m$ is the excitation coefficient and ${}_nC_l^m$ is the singlet spectrum. Since the spherical harmonics are orthogonal we form the stacked spectrum

$${}_nS_l^m(\omega) = \sum_j \bar{Y}_l^m(\theta_j, \psi_j) U(\omega, \theta_j, \psi_j).$$

where the overscore means complex conjugation. For an isolated multiplet we obtain

$${}_nS_l^m(\omega) = \sum_j \bar{Y}_l^m(\theta_j, \psi_j) \sum_{m'=-l}^{l} {}_nA_l^{m'} Y_l^{m'}(\theta_j, \psi_j)\, {}_nC_l^{m'}(\omega)$$

Dropping the n, l indices we have

$$S_m = \sum_{m'=-l}^{l} A_{m'} \left[\sum_j \bar{Y}^m(\theta_j, \psi_j) Y^{m'}(\theta_j, \psi_j) \right] C_{m'}(\omega)$$

For a large number of uniformly spaced stations the term in brackets approaches a surface integral which equals the Kronecker $\delta_{mm'}$ and $S_m = A_m C_m(\omega)$. Even for a small number of stations one singlet is added always in phase while the others are not and therefore tend to cancel.

Operationally, we must render our six seismograms directly comparable. Therefore, we have removed the instrument phase and normalised the instrument amplitude to a standard response[8]. Finally, we have selected a common time window leaving us with six 155-h records beginning 25 h after the event. Gilbert and Dziewonski[6] show how to avoid discarding data as we have done, but their method involves assumptions about attenuation which we are unwilling to make.

We have applied the spherical harmonic stacking procedure to the multiplet ${}_0S_3$ and representative results are shown in Fig. 4 for $m=-3, 0, 3$. The spherical harmonic stacking procedure has worked well. Using the moment ratio method[4] we have measured all $2l+1$ (that is, seven) singlet frequencies ω_m. This is the first time that a split multiplet has been completely resolved.

Let ω_d be the degenerate eigenfrequency in the absence of rotation and ellipticity of figure. (We ignore other perturbations.) The relation between ω_m and ω_d is

$$\omega_m = \omega_d(1 + a + mb + m^2c)$$

The perturbation parameter b arises from the first order effect of Coriolis force. The perturbation parameter c arises from ellipticity (c_e), the non-radial part of the rotation potential (c_n), and the second order effect of Coriolis force (c_2)

$$c = c_e + c_n + c_2$$

The perturbation parameter a has, in addition,

$$a = a_e + a_n + a_2 + a_r$$

a term a_r from the radial part of the rotation potential. We have

$$a_s + \frac{l(l+1)}{3} c_s = 0 \quad \text{for } s = e \quad \text{and } n$$

Thus $\bar{\omega}$ the mean of the $(2l+1)\omega_m$ is

$$\begin{aligned}\bar{\omega} &= \frac{1}{2l+1} \sum_{m=-l}^{l} \omega_m \\ &= \omega_d\left(1 + a_2 + a_r + \frac{l(l+1)}{3} c_2\right) \\ &= \omega_d(1+p)\end{aligned}$$

From our observations of ω_m we can obtain $\bar{\omega}$, but to obtain ω_d we must have p, a functional of the Earth's structure, given the rate of rotation. Tables of a_s, b, and c_s have been prepared[5]. The value of p changes very little from one acceptable Earth model to another. We are probably justified, at this state of our knowledge, in using the tables to infer ω_d from $\bar{\omega}$. For ${}_0S_3$ we have computed ω_d and $\omega_m^{(\text{com})}$ from $\bar{\omega}$ and tabulated values of a_s, b, and c_s. The comparison is shown in Table 1.

The differences between $\omega_m^{(\text{com})}$ and $\omega_m^{(\text{obs})}$ have been used to compute a (relative) standard error of the mean (s.e.m.) of $\bar{\omega}$ of 1.7×10^{-4}. The perturbation parameter p seems to vary by less than 20% among the acceptable Earth models leading to a relative change in ω_d of less than 8.6×10^{-6}. Thus the relative error in $\bar{\omega}$ can safely be assigned to ω_d as well.

We have also applied the spherical harmonic stacking procedure to the ${}_0S_2$ multiplet. Cancellation is not as good as for ${}_0S_3$ but the frequency spacing between singlets is greater. Again we have completely resolved the multiplet, revealing two of the five singlets which were not visible in the original data. We have computed $\bar{\omega}$, ω_d, and $\omega_m^{(\text{com})}$ (as for ${}_0S_3$) and displayed them along with $\omega_m^{(\text{obs})}$ in Table 2. The relative error in $\bar{\omega}$ is 5.0×10^{-5}. A 20% change in p leads to a relative change in ω_d of 2.1×10^{-5}. Thus, it is reasonable to assign a relative error of 5.0×10^{-5} to ω_d.

The agreement between observed and computed singlet frequencies in Tables 1 and 2 is good evidence that the theoretical splitting formula[5] is correct and that model values of a, b and c are correctly computed and are close to the truth. From the data in Tables 1 and 2 we can determine a best fitting quadratic in m

$$\omega_m = \omega_0 + mf + m^2 g$$

by the method of least squares, where

$$\omega_0 = \omega_d(1+a); \quad f = \omega_d b; \quad g = \omega_d c$$

The results for ${}_0S_3$ are

$\omega_0 = 2.94523 \times 10^{-3} \quad \text{s}^{-1}$

$f = 1.378 \times 10^{-5} \quad \text{s}^{-1} \pm 0.78\%$

$g = -4.27 \times 10^{-7} \quad \text{s}^{-1} \pm 13\%$

where the errors are computed assuming that each $\omega_m^{(\text{obs})}$ is equally accurate. For comparison, model values are

$$f^{(\text{com})} = 1.360 \times 10^{-5} \quad \text{s}^{-1}$$
$$g^{(\text{com})} = -3.47 \times 10^{-7} \quad \text{s}^{-1}$$

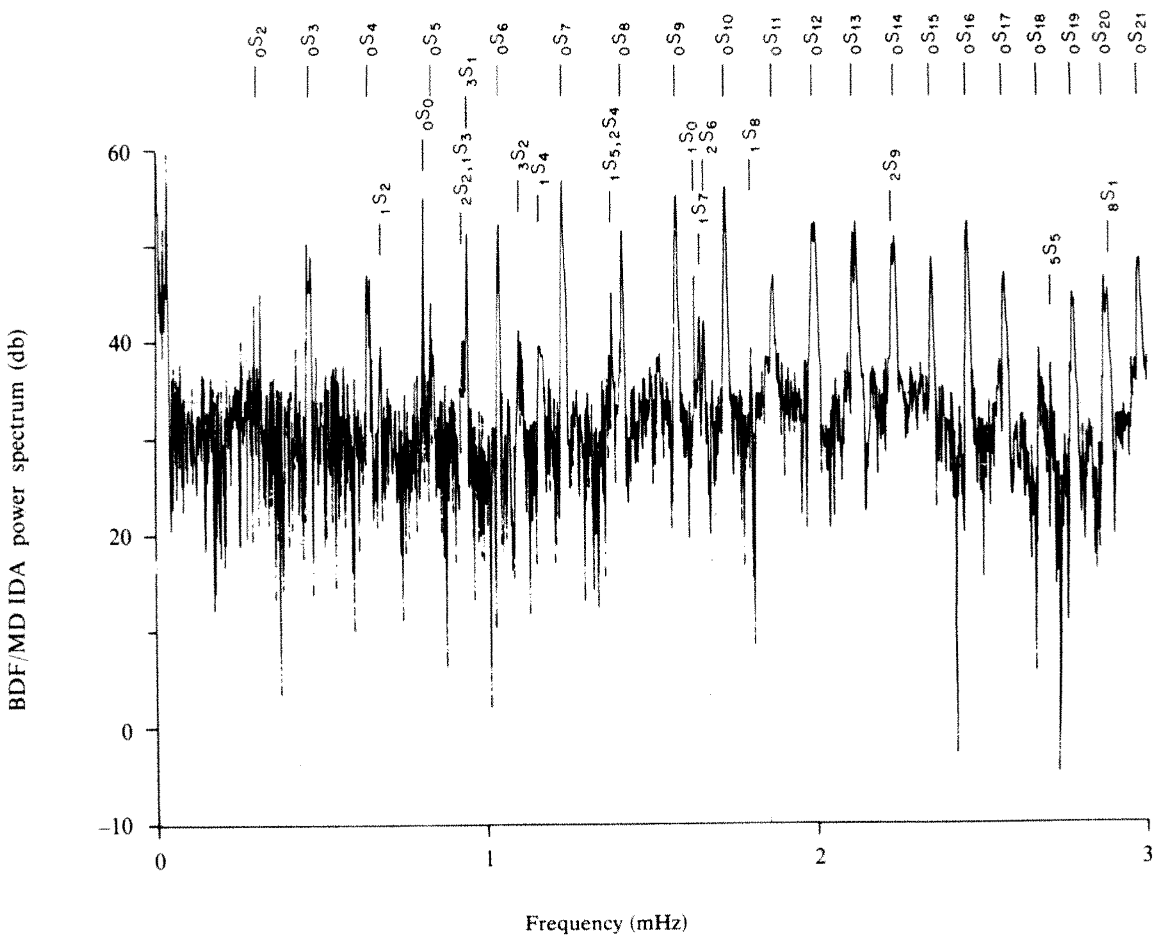

Fig. 2 A log power spectrum of 155 h of record from station BDF beginning 25 h after the event. Note particularly the good excitation of ${}_0S_0$, and many overtones as well as the complexity (due to splitting) of the lowest order fundamental modes: ${}_0S_2$, ${}_0S_3$, and ${}_0S_4$.

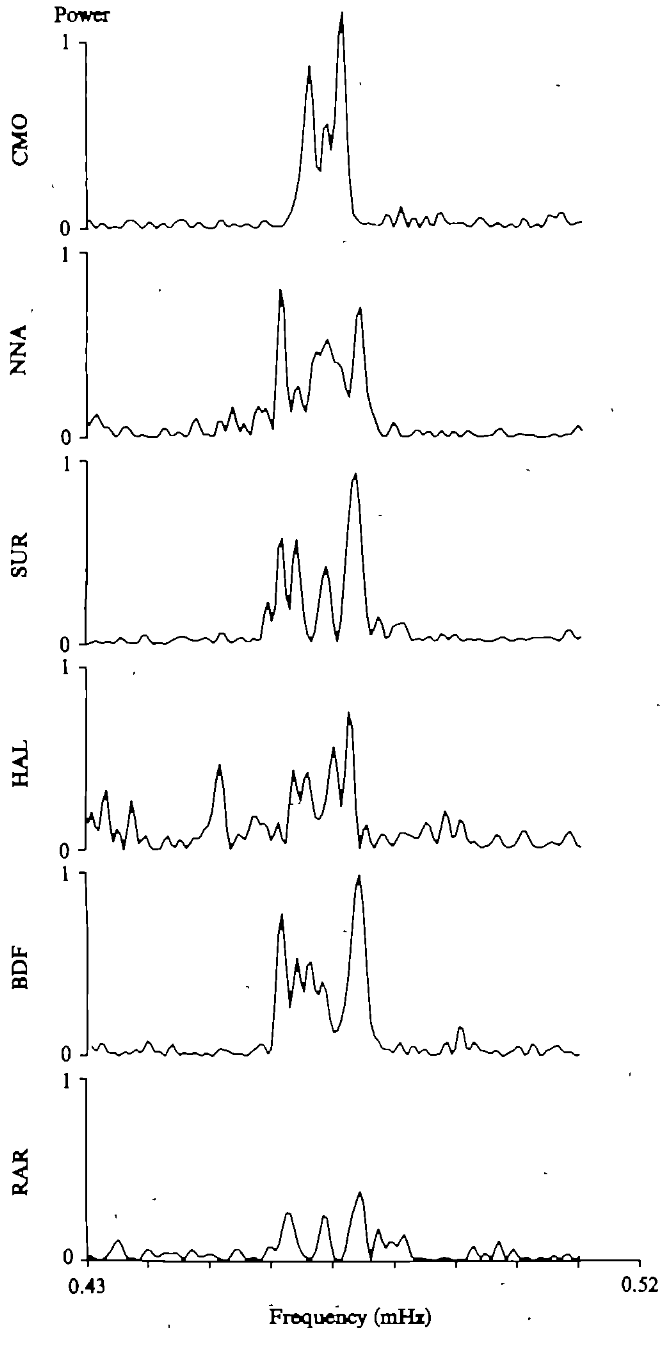

Fig. 3 Interpolated linear power spectra (arbitrary units) of the fundamental mode $_0S_3$ for each of the six stations used. In each case, 155 h of record beginning 25 h after the event were used.

The difference between computed and observed values of f is 1.7σ or 1.3%. For g the difference is 1.8σ or 23%.

The results for $_0S_2$ are

$$\omega_0 = 1.94445\times10^{-3} \quad \mathrm{s}^{-1}$$

$$f = 2.9015\times10^{-5} \quad \mathrm{s}^{-1} \pm 0.46\%$$

$$g = -3.407\times10^{-7} \quad \mathrm{s}^{-1} \pm 31\%$$

The computed[5] values of f and g are

$$f^{(\mathrm{com})} = 2.8973\times10^{-5} \quad \mathrm{s}^{-1}$$

$$g^{(\mathrm{com})} = -5.192\times10^{-7} \quad \mathrm{s}^{-1}$$

The difference between computed and observed values is 0.3σ or 0.14% for f and 1.7σ or 52% for g.

The computed and observed values of f and g are not significantly different at the 2σ level. However, at the 1σ level, only f for $_0S_2$ shows no significant difference. If further research supports the existence of significant differences between observed and computed values of splitting parameters, then the observed values represent new constraints on models of the structure of the Earth. For example, the Fréchet derivative of f with respect to structure is a functional only of the density distribution. Presumably, if one could observe numerous Coriolis splitting parameters, f, one could obtain improved constraints on the density distribution within the Earth.

Table 1 The singlets of $_0S_3$

m	$\omega_m^{(\mathrm{obs})}\times10^3$ s^{-1}	$\omega_m^{(\mathrm{com})}\times10^3$ s^{-1}
−3	2.90076	2.90114
−2	2.91590	2.91628
−1	2.92903	2.93085
0	2.94575	2.94474
1	2.95881	2.95806
2	2.97301	2.97075
3	2.98137	2.98275
	$\bar{\omega} = 2.94352\times10^{-3}$ s^{-1}	$p = 4.3\times10^{-5}$
	$\omega_d = 2.94339\times10^{-3}$ s^{-1}	s.e.m. $= 5.0\times10^{-7}$ s^{-1}

Attenuation

There are surprisingly few really precise data concerning the anelasticity (or Q) of the Earth. While we plan to measure the Q values of all visible modes in our Indonesian data set, for this preliminary report we have decided to concentrate on two unusually interesting modes: $_0S_0$ and $_1S_0$.

The radial $_nS_0$ modes store strain energy primarily in compression, rather than shear[9], and have large Q values. The modes $_0S_0$ and $_1S_0$ in particular have less shear energy than the other radial modes and the measurement of their extremely high Q values has proved to be elusive. Our data set affords us the unique opportunity not only to measure these Q values, but to explore the reproducibility of the results. Our procedure, therefore, will be to examine the six records separately for consistency, and derive our final Q value from the spherical harmonic stack. We measure α_0 where $\alpha_0 = \omega_0/2Q$.

Because of the high Q values of these modes the width of the peak due to record length is large compared with the width due to attenuation for the record length we have. In order to use a Q estimator based on measuring width it is necessary to make the width due to attenuation comparable with the width due to record length (they are equal at 0.885 Q cycles). Conservatively we usually use 1.5Q cycles or over 2,000 h in the case of $_0S_0$. Even at that we would have lost only 3 db over the maximum attainable S/N (at about 560 h according to Buland and Gilbert[4]). This means that such a measurement would be possible, but we would need to edit another 1,500 h of data on each station. For the data set at hand our experiments have shown that a simple time lapse method works as well as anything. We measure the integrated power between half power points in windows lagged so as to have no overlap.

This is a delicate task. To enhance our statistics we need as many windows as possible. But if each window is too short the signal will be overcome by noise. Further, the total time series length T must be long enough so that the mode has decayed somewhat to gain significance. But, if T is too large the latter windows again will have an unacceptable S/N.

Table 2 The singlets of $_0S_2$

m	$\omega_m^{(\mathrm{obs})}\times10^3$ s^{-1}	$\omega_m^{(\mathrm{com})}\times10^3$ s^{-1}
−2	1.88502	1.88489
−1	1.91511	1.91518
0	1.94458	1.94471
1	1.97292	1.97317
2	2.00119	2.00082
	$\bar{\omega} = 1.94376\times10^{-3}$ s^{-1}	$p = -1.04\times10^{-4}$
	$\omega_d = 1.94396\times10^{-3}$ s^{-1}	s.e.m. $= 9.8\times10^{-8}$ s^{-1}

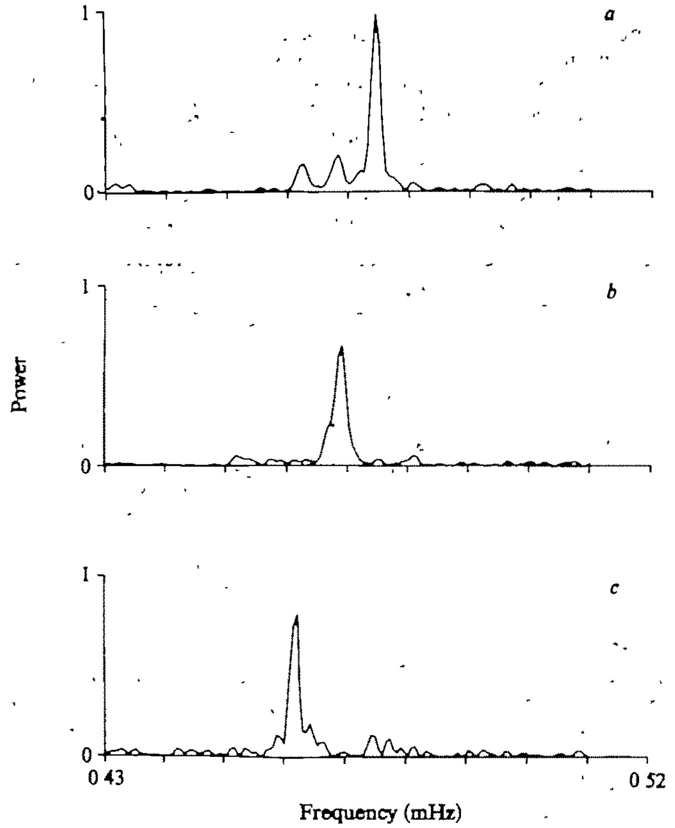

Fig. 4 Interpolated linear power spectra (arbitrary units) of stacks for *a*, ${}_0S_3^{+3}$; *b*, ${}_0S_3^0$; *c*, ${}_0S_3^{-3}$. All seven ${}_0S_3^m$ singlets were resolved.

As a result of these trade offs, we have chosen for ${}_1S_0$ 10^5-s windows in a 150-h series and for ${}_0S_0$ 2×10^5-s windows in as long a series as we can get. Therefore, for ${}_1S_0$ we may work with the six 155-h records described above. But for ${}_0S_0$ we will have to do something more elaborate for a stack, as not all the available records are of sufficient length and some of the records have large data gaps. Because of these gaps a straightforward deconvolution of the series to remove the instrument response would distort good data points adjacent to the gaps. The procedure adopted to avoid this was to correct for instrument response at only one frequency by multiplication of the time series by a complex number that is the reciprocal of the instrument response at the desired frequency. Then time series were averaged point by point with data gaps not included in the average, to form a 575-h stack with few data gaps.

For ${}_1S_0$ we used five windows. The Q results for individual records scatter approximately 50% about their mean with relative errors of the least squares fit ranging between 13 and 63%. Our final value is $Q=2{,}360\pm15\%$, obtained from a 150-h stack. Our final error estimate is the maximum likelihood value derived from the single station measurements. On the basis of the individual record Q values, the final error bound seems to be rather small (it is based on only six data), but not totally unreasonable.

For ${}_0S_0$, the five individual records are of various lengths yielding up to fifteen 10^5-s windows (avoiding gaps). Individual record Q values scatter as much as 80% about their mean with relative errors of the least squares fit ranging between 13 and 105%. For the final (stack) result we used six 2×10^5-s windows (to boost S/N). The result is: $Q=4{,}100\pm26\%$.

Dahlen[10] has extended the work of Munk and Hasselmann[11] on the expected quality of spectral parameters measured in the presence of noise. He considered the linear, least-squares estimation of the (complex) frequency of a decaying sinusoid and obtained expressions for the variance

$$\langle\delta\omega_0^2\rangle=\langle\delta\alpha_0^2\rangle=\frac{R(2\alpha_0T)}{T^2(S/N)}$$

The function $R(x)$ is well approximated by $R(x)=2(x^2+9)^{1/2}$. S/N is the ratio of peak signal to average noise in the power spectrum and T is record length.

Let $\sigma_D^2=\langle\delta\omega_0^2\rangle=\langle\delta\alpha_0^2\rangle$. Our result for ${}_1S_0$ gives

$$\sigma_D=5.0\times10^{-7}\ \mathrm{s}^{-1};\qquad \sigma_D/\alpha_0=23\%$$

for $S/N=20$ db in the radial stack. The assigned relative error of 15% seems reasonable. In the case of ${}_0S_0$ we have

$$\sigma_D=5.4\times10^{-8};\quad \sigma_D/\alpha_0=9\%$$

for $S/N=28$ db in the radial stack. Again, the assigned relative error of 26% seems reasonable.

Frequencies

The observations of all singlets in the multiplets ${}_0S_2$ and ${}_0S_3$ allowed us to infer the gross Earth data, ω_d, for these modes with greatly improved precision:

$$\omega({}_0S_2)_d=1.94396\times10^{-3}\pm9.8\times10^{-8}\ \mathrm{s}^{-1}$$

$$\omega({}_0S_3)_d=2.94339\times10^{-3}\pm5.0\times10^{-7}\ \mathrm{s}^{-1}$$

From the six individual (155-h) records we have measured frequencies for the radial modes ${}_0S_0$ and ${}_1S_0$ with the following results and standard errors of the mean:

$$\omega({}_0S_0)=5.11850\times10^{-3}\pm1.5\times10^{-7}\ \mathrm{s}^{-1}$$

$$\omega({}_1S_0)=1.02532\times10^{-2}\pm1.0\times10^{-6}\ \mathrm{s}^{-1}$$

As $l=0$ there is only one singlet for each of these modes. However, a computed correction must be made to find ω_d as above. The results are:

$$\omega({}_0S_0)_d=5.11679\times10^{-3}\pm1.5\times10^{-7}\ \mathrm{s}^{-1}$$

$$\omega({}_1S_0)_d=1.02498\times10^{-2}\pm1.0\times10^{-6}\ \mathrm{s}^{-1}$$

The standard errors assigned to the radial modes are in agreement with the criterion of Dahlen. So are those of ${}_0S_2$ and ${}_0S_3$ provided we take into account the number of singlets in each multiplet and accept theoretical values for α_0. It would be more satisfactory to use observed values of α_0 and we intend to obtain them.

Discussion

The IDA network has given us the capability to refine our studies of splitting and attenuation, and to improve significantly the resolvability of spectral parameters in the low frequency seismic band. At the time of the Indonesian earthquake there were nine IDA stations, six of which provided the records used in this article. Now there are 14 stations with an additional three scheduled for installation within a year. With an enlarged network we should be able to resolve multiplets of higher order, and improve the accuracy of our measurements of ω_0 and α_0. Improved measurements will lead to greatly improved models of attenuation and laterally heterogeneous structure of the Earth.

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letters

Evidence of a disrupted pulsar binary system

THIS letter demonstrates that the pulsars PSR1952+29 and PSR1929+10 may have originated in a binary system which was disrupted by a supernova explosion less than a million years ago. If further confirmed by improved observations, this result provides an evolutionary link between the separate phenomena of X-ray binary systems and radio pulsars.

PSR1952+29 has a characteristic age of 3,600 Myr as measured by the usual ratio $\tau = P/2\dot{P}$ (where P is the pulsar period and $\dot{P}$ its time derivative). This is the largest known value. PSR1929+10 has a characteristic age of 3.1 Myr, a more typical value. The characteristic age of a pulsar is generally regarded as an overestimate of its true age, and independent estimates[1] based on kinematic arguments suggest that pulsars have a true average age of 2 Myr.

Recent results[2] have given the distance to PSR1952+29 as 240 pc, and its closeness to Earth has made it possible to detect its proper motion (200 km s^{-1}) in both magnitude and direction. Gullahorn and Rankin[3] report this together with similar measurements of PSR1929+10 and six other pulsars. PSR1929+10, whose galactic coordinates are about 20° from PSR1952+29, is estimated to be at a distance of 110 pc and to have a proper motion of 85 km s^{-1}. Both pulsars lie close to the galactic plane (their galactic coordinates are respectively $l^{II} = 47.4°$, $b^{II} = -3.9°$ and $l^{II} = 65.9°$, $b^{II} = 0.8°$), with PSR1952+29 actually having a small velocity component into the plane. The velocity vectors of both pulsars point away from a region of the sky from which they can both conceivably have come within a pulsar lifetime. This leads one to suspect that they began their lives as pulsars in a common event. To test this hypothesis it is possible in principle to determine the coordinates of the coincidence from the coordinates and transverse velocities of the pulsars, then to check that both pulsars were at this point at the same time. In practice, errors in the distances and coordinate velocities of the pulsars will mean that the coincidence is never exact. Here we adopt the procedure of choosing possible galactic coordinates for the coincidence (the best fit was given by $l_0^{II} = 32°$, $b_0^{II} = 3°$) and computing the velocity which the pulsars would have at their present coordinates if they had started simultaneously from this point. Writing the velocities of PSR1929+10 and PSR1952+29 in galactic coordinates as (v_{l_1}, v_{b_1}) and (v_{l_2}, v_{b_2}) respectively, we obtain the fit

$$v_{l_1} = 80\ \mathrm{km\,s^{-1}}\ (78) \qquad v_{b_1} = -35\ \mathrm{km\,s^{-1}}\ (-30)$$

$$v_{l_2} = 167\ \mathrm{km\,s^{-1}}\ (198) \qquad v_{b_2} = -12\ \mathrm{km\,s^{-1}}\ (-39)$$

The observed[3] values being given in parentheses. The computed values lie within the error bars of the observations. For this calculation it is assumed that the pulsars have moved in straight lines in space, neglecting the gravitational force of the Galaxy (which is a second-order effect for such fast-moving objects). If the two pulsars were coincident at a distance r_0 at a time T ago, then the above fit of their present projected motions on the celestial sphere is given by taking the ratio r_0/T as 300 km s^{-1}. In the absence of measured radial velocities, r_0 and T cannot be determined independently from the transverse motions. If, however, we impose the condition that the radial and transverse velocity components are comparable, then a possible choice of parameters is $T = 600{,}000$ yr, $r_0 = 180$ pc, which yields $v_{r_1} = -106$ km s^{-1}, $v_{r_2} = +152$ km s^{-1}.

Most supernova remnants have estimated ages <0.1 Myr (ref. 4). It is usually assumed that after this time they merge with the surrounding interstellar medium[5]. The remnant of the supernova taking place 0.6 Myr ago would now, if detectable at all, be a large diffuse area over 30 pc across[6]. At a distance of 180 pc this would have a diameter of at least 10°. Radio maps[7] do not reveal any such feature centred on the suggested coincidence. However, it is possible to discern a roughly circular feature with a diameter about 7° centred at $l^{II} = 33°$, $b^{II} = 12.5°$. Although fresh data on proper motions could bring about a readjustment of the point of coincidence, an association with this feature must be regarded as highly tentative.

Nevertheless, on the basis of the calculations here it seems possible to suppose that pulsars PSR1929+10 and PSR1952+29 emerged from a binary system which was disrupted by an explosive event ~0.6 Myr ago. As it has the younger characteristic age, one may reasonably assume that PSR1929+10 is the neutron star which was formed in the explosion. Before this, PSR1952+29 will have existed as a neutron star in orbit around a more massive companion which eventually gave birth to PSR1929+10. If confirmed by further measurements, this result provides evidence for van den Heuvel's account[8] of the final phase of binary evolution and supports the suggestion[9–12] that pulsars originate in close binary systems.

Without going into the details of close binary evolution here, we can imagine that the binary experience had two important effects on the neutron star which became PSR1952+29. First, having itself been formed in a supernova explosion between 1 and 10 Myr previously[8], its magnetic field would have had time to decay from its original strength before it was released as a single pulsar. Making the conventional assumption that a pulsar's magnetic field is proportional to $(P\dot{P})^{1/2}$, we obtain a value of 3×10^{10} G for PSR1952+29, compared with 5.2×10^{11} G for PSR1929+10 and 3.8×10^{12} G for the recently-born Crab pulsar. Second, if PSR1952+29 has passed through a spin-up phase (as an X-ray pulsar), then it will have accreted angular momentum from its companion. Its present low braking rate (presumably the consequence of a low magnetic field) implies that its period (0.43 s) has not changed significantly since the break-up of the binary system, but before this both considerable spin-up and spin-down will probably have occurred[12].

A histogram[13] of the characteristic ages of the 89 pulsars with measured $\dot{P}$ reveals that while 68 of them (including PSR1929+10) belong to a convincing power-law distribution tailing off at 20 Myr, an additional 19 pulsars (including PSR1952+29) do not fit this pattern, having anomalous characteristic ages ranging from 22 to 3,600 Myr. The result here strongly implies that the 'anomalous' pulsars have spent a significant part of their lives as neutron stars in binary systems. The existence of two populations of pulsars arising in this way has been suggested by several authors[10–12]. Here we emphasis that the magnetic field will be the factor which distinguishes between them. Precisely this classification of pulsars was made by Helfand and Tademaru[10].

As the experience of PSR1952+29 is not likely to be unique,

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Table 1 Examples of possibly 'paired' pulsars

S (pc)	Pair	d (kpc)	P (s)	τ (Myr)
7	1700−18	0.2	0.80	?
	1706−16	0.2	0.65	1.6
18	0809+74	0.2	1.29	128
	0904+77	?	1.59	?
52	1604−00	0.4	0.42	22
	1642−03	0.2	0.39	3.4
91	2106+44	0.8	0.42	66
	2021+51	0.8	0.53	2.7
100	2324+60	0.8	0.23	10
	2223+65	0.7	0.68	1.1
140	1426−66	2.4	0.79	?
	1449−64	2.6	0.18	?
213	1717−29	1.6	0.62	12
	1730−22	1.6	0.87	?
250	0138+59	3.0	1.22	97
	0105+65	1.0	1.28	1.6
300	2305+55	1.6	0.46	75
	2217+47	1.6	0.54	3.1
450	1819−22	5.7	1.87	49
	1754−24	6.3	0.23	?

S is the approximate transverse separation of the pairs; d, distance to each pulsar; P, period, and τ the characteristic age (data from ref. 13).

it will be of interest to investigate the origins of the remaining 18 anomalous pulsars to see if they have previously unrecognised companion pulsars with whom they once formed binary systems. Some examples of possible 'pairs' are given below in order of increasing transverse separation. Since a pulsar with velocity 200 km s^{-1} travels 200 pc in 1 Myr it is extremely difficult to recognise pairs without accurate velocity vectors, and this list is little more than informed guesswork. The pairs have been chosen on the basis that both pulsars are at roughly the same distance from Earth and are 'reachable' from one another. Assuming the pulsars in each pair have once formed a binary system we have given them in expected order of formation as neutron stars.

The pair PSR1700−18 and PSR1706−16 seems interesting because of their closeness to one another and, as elsewhere on the list, one would at least like to see the question-mark eradicated. Both PSR0809+74 and PSR0904+77 are to be found in a high latitude 'hole' in the galactic HI distribution[14]. The association of the 'hole' (presumably a supernova remnant) with PSR0809+74 has been regarded as suspect on account of the latter's large apparent age. It could be that PSR0904+77 is the missing younger companion. A further list of possible pairs, chosen on a similar basis, is given in ref. 9.

Finally, one should consider the future of PSR1952+29 and PSR1929+10. The characteristic age of the latter shows that it is slowing down on a timescale roughly comparable with pulsar lifetimes and it will presumably switch off when larger values of P are reached. This argument is not, however, available for PSR1952+29. Its emission may fade through the decay of its already low magnetic field, but the lifetimes of this and the other anomalous pulsars need not bear any relation to the typical lifetimes of their former companions.

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Brightest members of clusters of galaxies

THERE are two opposing interpretations of the small dispersion $\sigma_1 \sim 0.35$ mag in the absolute magnitudes M_1 of the brightest members of rich clusters of galaxies measured by Sandage[1]. In one view, advanced, among others, by Peebles[2,3], it is assumed that there is a universal cluster luminosity function, and that the value of σ_1 and of the variation of M_1 with cluster richness can be explained as the statistical result of drawing the brightest member from a set of clusters obeying this function. There is therefore nothing physically remarkable about the brightest member, and its luminosity is determined by the same mechanism as that which determines the luminosity of the fainter members. The opposing view, which has been advanced by Peach[4], Sandage[1], and Tremaine and Richstone[5], claims that the small dispersion is due to special conditions governing the formation or evolution of the brightest member, and is therefore entirely or in part independent of the luminosity function of the other cluster members. On this view one evokes either special conditions at the epoch of galaxy formation[1], or a mechanism, such as galactic accretion[6], which governs the size and luminosity of the most massive galaxy in such a way as to give a small dispersion in the metric magnitude. The weakness of the statistical model, according to its opponents, is its inability to account for σ_1 without postulating a luminosity function steeper than those directly observed. The opposing view is open to the criticism that it is either *ad hoc* or that the mechanisms of galaxy evolution are too uncertain to lead to reliable conclusions. Here, we discuss the two interpretations in the light of new results and suggest that the case against the statistical model may not be as strong as sometimes suggested.

During a programme of photometry of clusters, we have measured the absolute isophotal magnitudes of the brightest members of the 12 rich clusters listed in Table 1. Details of the methods of observation and reduction are the same as those used in our previous work[7–9]; full lists of magnitudes will be published elsewhere, except for A1413, A1656 and A1930, which are already available. After the cluster name we give the redshift z, the cluster richness R, the radius r_{25}, defined as $(a/\pi)^{1/2}$ where a is the area contained within the standard

Table 1 Isophotal magnitudes for the 3 brightest members of 12 rich clusters of galaxies

Cluster	Redshift	Richness class	Isophotal radius (kpc)	Absolute V magnitudes for the 3 brightest cluster members		
	z	R	r_{25}	M_1	M_2	M_3
Virgo	0.0038	0	43	−22.9	−22.4	−22.4
A426	0.0181	2	41	−24.1	−23.5	−23.3
A1377	0.0516	1	32	−22.9	−22.8	−22.2
A1413	0.1426	3	61	−24.6	−22.6	−22.6
A1553	0.1651	2	54	−24.1	−24.0	−23.7
A1656	0.0228	2	47	−23.9	−23.5	−23.3
A1930	0.1312	1	39	−23.4	−22.6	−22.2
A2065	0.0722	2	32	−22.8	−22.7	−22.5
A2147	0.0351	1	42	−23.2	−22.9	−22.6
A2151	0.0341	2	37	−23.0	−22.9	−22.8
A2199	0.0303	2	40	−23.9	−22.7	−22.6
A2670	0.0775	3	48	−23.9	−22.9	−22.9

isophote for the brightest member, and absolute *V* magnitudes M_1, M_2 and M_3 for the first, second and third brightest cluster member measured to the 25 mag arc s^{-2} isophote ($H_0 = 50$ km s^{-1} Mpc^{-1}; $q_0 = +1$). Table 2 gives the dispersions in M_1, M_2 and M_3 for the sample.

The absolute magnitudes M_1 of Sandage, on which the entire controversy has until now been based, have been derived by fitting photoelectric magnitudes measured through a series of circular apertures to a standard growth curve of magnitude against radius from the galaxy centre[10]. The small scatter of the observed points about the growth curve is claimed as evidence that the light distributions of the galaxies in his sample are similar to radii of ~40 kpc. The light contained within a standard metric radius of 43 kpc at the galaxy is inferred from the curve and gives, after absorption and K-corrections, the final metric magnitude. The second and third brightest, M_2 and M_3, were not measured directly in this way, but their magnitudes were calculated on the assumption that the ratio of their luminosities to that of the brightest is given by the ratio of the apparent sizes of their images to that of the brightest; M_2 and M_3 are thus isophotal magnitudes. The dispersions σ_1, σ_2 and σ_3 for Sandage and Hardy's[11] magnitudes for the 78 clusters in their sample and for the 12 clusters of Table 1 are shown in Table 2. It will be seen that the results for the present sample are not significantly different from those for the larger one. It will also be clear that $\sigma_1 = 0.57 \pm 0.18$ for the isophotal magnitudes is about one standard deviation larger than that for the metric magnitudes $\sigma_1 = 0.38 \pm 0.12$, whereas σ_2 and σ_3 are essentially the same. This difference cannot be attributed to measurement errors; we estimate our errors at about 0.2 mag which would imply a corrected dispersion $\sigma_1 = 0.53$ mag.

The reason for the greater spread in M_1 measured to a fixed isophote rather than to a fixed radius can be understood in the following way. The size of the brightest member to the 25 *V* mag arc s^{-2} isophote r_{25} ranges from 32 kpc in A1377 to 61 kpc for the cD galaxy in A1413, with a mean of 42 kpc, fortuitously close to Sandage's standard metric radius. This radius is correlated with the isophotal magnitude in the sense that brighter galaxies tend to be larger, and fainter galaxies smaller, than the mean. As metric magnitudes are measured to a constant radius close to our mean, bright galaxies will possess brighter isophotal magnitudes than metric, whereas faint galaxies will possess fainter isophotal magnitudes, because of the difference in areas contributing to the integrated luminosity in the two methods of measurement. We find that for our sample

$$M_1 = -(7.3 \pm 1.5) \log r_{25} + \text{constant} \qquad (1)$$

Sandage[10] finds a dispersion in isophotal diameters, *d*, for 27 brightest members, after correction for measuring errors,

$$\sigma(\log d) = 0.075 \pm 0.02 \qquad (2)$$

close to the dispersion of our sample 0.080 ± 0.025. Our empirical relationship (1) when combined with (2) leads to a dispersion in the isophotal magnitudes of Sandage's sample of 0.54 mag which is close to our value. We have found that for reasonable functional forms of the radial luminosity distribution (1) is a likely consequence of correlations between the central surface brightness and the isophotal magnitude and between the scale-length of the distribution and magnitude, in the sense that brighter galaxies possess both higher central surface brightness and flatter luminosity profiles than fainter ones. There is some direct evidence for this in the work of Oemler[12].

Geller and Peebles[3] have calculated in detail the predictions of the statistical model using a luminosity function based on Abell's observations of four rich clusters; this function also agrees well with one we have derived for the clusters of Table 1. Table 2 gives their values for σ_1, σ_2 and σ_3. Note that there is good agreement with our isophotal results but a disagreement with σ_1 for the metric magnitudes. They further predict a linear dependence of M_1 on richness class *R* with a slope of $dM_1/dR = -0.2$ mag per class. Sandage[1] has shown that for his larger sample the actual variation must be much flatter. In our present sample of clusters, which is too small to give a good enough value for the slope to enable a direct comparison with the theory, $dM_1/dR = -0.21 \pm 0.25$ for the metric magnitudes and $dM_1/dR = -0.47 \pm 0.31$ for the isophotal. The latter is more than one standard deviation greater than zero and, if confirmed with a larger sample, would be difficult to explain other than on a statistical model.

Table 2 Dispersions in the absolute *V* magnitudes of the 3 brightest members

Magnitudes		σ_1	σ_2	σ_3
Isophotal magnitudes from this work		0.57 ± 0.18 (12)	0.45 ± 0.14 (12)	0.45 ± 0.14 (12)
Metric M_1 and isophotal M_2 and M_3 from Sandage and Hardy[11]	All clusters	0.34 ± 0.04 (73)	0.51 ± 0.06 (74)	0.55 ± 0.06 (74)
	Clusters of Table 1	0.38 ± 0.12 (12)	0.49 ± 0.17 (10)	0.48 ± 0.17 (10)
Predictions of the statistical model (Geller and Peebles[3])		0.53	0.48	0.47

Errors are standard deviations. The number of galaxies in each sample is given in parentheses.

The discussion of the statistical model has been carried further by Tremaine and Richstone[5], who have derived two tests of the model which are more general than those of Geller and Peebles, in that they are independent both of the assumed form of the luminosity function and of its uniformity from cluster to cluster. Assuming solely an asymptotic behaviour of cluster luminosity functions certainly true of all observed functions, they prove that the two parameters

$$t_1 = \sqrt{\frac{N}{N-1}}\,\frac{\sigma_1}{\langle\Delta\rangle} \quad \text{and} \quad t_2 = \sqrt{\frac{N}{N-1}}\,\frac{\sigma_\Delta}{(0.677)^{1/2}\langle\Delta\rangle}$$

must be $\geqslant 1$ for any large sample of clusters. Here, $\Delta = M_2 - M_1$, σ_Δ is its dispersion, $\langle\Delta\rangle$ its mean, and *N* the number of clusters. Their application of the test using the Sandage and Hardy magnitudes gave values of t_2 near unity, but t_1 was significantly less than unity for every sub-set of the data examined. Their conclusion was that no statistical model was likely to be valid. Using the isophotal magnitudes of Table 1, $t_1 = 1.0$ and $t_2 = 1.2$, a result consistent with the model. The change is mainly due to the increase of σ_1. Although the present sample is small, the result again supports the view that the use of isophotal magnitudes may remove the discrepancy between observation and theory.

If the isophotal magnitude is a physically more significant parameter than metric magnitude, being more closely related to the mass of the galaxy, and if a larger sample of clusters confirms our result that the consistent use of isophotal magnitudes removes much of the conflict between the statistical model and the observations, it does not necessarily imply that there is nothing unique about the properties of the brightest member, only that any integrated photometric differences it may have from other cluster members do not exceed limits set by the model. It is possible that cD galaxies are structurally different from other giant ellipticals. There is also a suggestion that there is a steepening of the luminosity function in the denser parts of clusters with particularly bright brightest members, such as the Coma cluster[9], similar to that predicted for galactic accretion; this is borne out in some of the luminosity functions of other clusters of Table 1. However, accretion on the brightest galaxy cannot have proceeded to the point where the galaxy has lost all those properties which are continuous with the properties of the fainter members.

If one accepts these conclusions, it is particularly difficult to maintain the view[1,4] that the smallness of σ_1 is in some way due to special conditions not applicable to other members at the time of galaxy formation.

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Nucleation and growth studies by small angle neutron scattering and results for a glass ceramic

THE nucleation of phase separation in bulk solids remains one of the more elusive phenomena in both experimental and theoretical terms[1]. Experimentally, the difficulties centre on the problem of gaining detailed structural information about the small composition fluctuations from which the precipitate develops. Theoretically, progress has been made in calculating stable microstructures in simple materials such as the rare gases[2] and amorphous metals[3] as well as identifying transient clusters in liquids which are precursors to long range order and thus may act as nucleation embryos[4]. However, it remains a formidable problem to relate such calculations to the microstructure of a multicomponent solid or liquid. The most readily observed physical property of nuclei is their ability to facilitate growth of a new phase in appropriate conditions. Consequently, the study of the growth process immediately following nucleation is the most accessible though indirect method of inquiry into nucleation. It is, however, often difficult to follow the very early stages of growth either because it occurs too rapidly, or because the size or total mass of the precipitate falls outside of the conventional range of structural techniques. We report here the striking effects of nucleation as revealed by a neutron small angle scattering study (SANS) of a glass ceramic ($2MgO$, $2Al_2O_3$, $5SiO_2 + 10\%$ TiO_2 nucleating agent) during the early stages of growth of the precipitate. As a result of the continuous viscosity–temperature relationship, the rate of phase separation in glasses can be put on a favourable time-scale to follow the kinetics of growth simply by working at the appropriate temperature. Also as will be evident below, neutron small angle scattering provides important advantages in following the growth of precipitates in bulk. Three particular advantages have been exploited. (1) The range in scattering vector $\mathbf{Q}(\mathbf{Q} = (4\pi \sin\theta)/\lambda)$ which it is possible to study includes the region around $\mathbf{Q} = 0.005$ Å^{-1} which generally falls between the scope of X-ray small angle scattering and light scattering measurements; (2) the low absorption of neutrons even at long wavelengths (~10 Å) allows the easy investigation in transmission of a bulk glass which is heated directly in the neutron beam; and (3) the precipitating phase (an aluminium titanate solid solution) is rich in titanium atoms, and the negative scattering length of titanium ensures that there is good scattering contrast between the precipitate and the host silicate glass. All the experiments were carried out at the Institut Laue-Langevin on the D11 spectrometer[5].

The general form of the scattering curves obtained is shown in Fig. 1; these curves were obtained on a glass nucleated by a pretreatment at 720 °C for 10 h. SANS is negligable immediately after nucleation and corresponds to the zero-time curve of Fig. 1. On heating *in situ* at 835 °C, SANS increases rapidly with the growth of the particles giving sufficient scattered intensity to allow the scattering curve to be followed every few minutes.

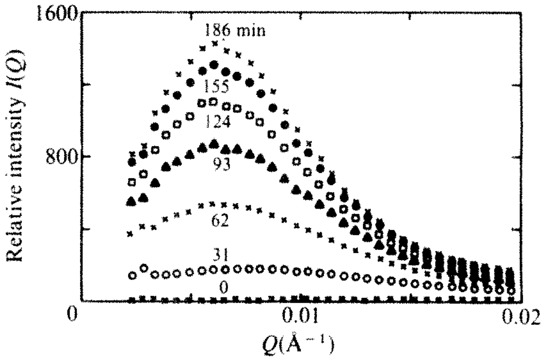

Fig. 1 Small angle neutron scattering curves from cordierite glass heated at 835 °C after nucleation pretreatment at 720 °C for 10 h.

We emphasise some features of these curves which are characteristic of the particle nucleation condition before the onset of growth. The dominating feature of the curves is the pronounced maximum ($Q_M = 0.007$ Å^{-1}) with the intensity falling towards zero at low Q. Even at the earliest times for which the precipite is very small, the maximum is apparent and the position remains stable during the growth period. The maximum is due to an interference effect between particles for which the spatial distribution is partly ordered. Similar effects are observed for the decomposition of alloys[6]. From the position of the maximum one obtains a rough estimate of the mean distance between the precipitates ($l \sim 2\pi/Qm$) of 900 Å corresponding to a precipitate density of about 10^{15} cm^{-3}. We follow the development of the precipitates using Guinier plots on the high Q side of the maximum. The form of the scattering curve is, however, distorted by the interference term, so that the values obtained in this way are only approximate. Within the limit of validity $QR_g \leq 1.2$ the scattering function $\overline{SQ}$ is related to the radius of gyration R_g according to $\overline{SQ} = \exp(-Q^2/R_g^{2/3})$ and $R_g = (^3/5)1/2R_s$, where R_s is radius of a spherical particle.

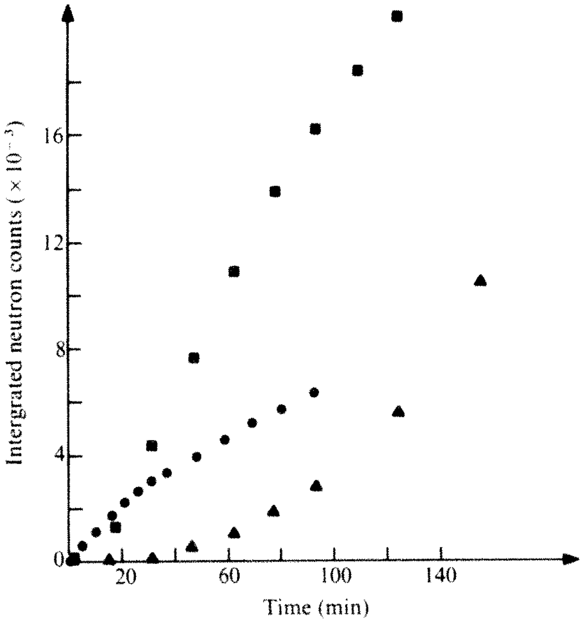

Fig. 2 Growth of integrated SANS intensity with time at 835 °C for different nucleation conditions. ■, 720 °C for 10 h; ●, 720 °C for 20 h; ▲, no nucleation treatment.

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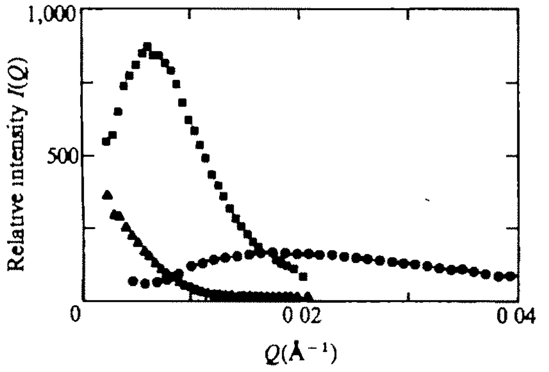

Fig. 3 SANS from cordierite glass heated for 90 min at 835 °C for different nucleation conditions. ■, 720 °C for 10 h; ●, 720 °C for 20 h; ▲, no nucleation treament.

For the glass discussed above, R_s increases from 40 Å after 2 min to 210 Å after 180 min at 835 °C. There is an initial period of about 10 min (the induction period) where the SANS intensity increases very slowly, followed by a longer period (100 min) during which a rapid linear increase of intensity with time occurs before the rate slowly declines (Fig. 2). The intensity is a function of the volume of the precipitate and the contrast between the precipitate and the glassy matrix, which increases during growth as a result of the depletion of titanium ions from the glass. Indeed we observe that the intensity is proportional to the square of the volume of the precipitate which leads to the result that the scattering contrast is directly proportional to the degree of depletion of the glassy matrix.

When the nucleation pretreatment time is varied, different scattering behaviour occurs (Fig. 3). Allowing no nucleation time we observe an induction period of ~50 min (Fig. 2), when weak scattering occurs in the low Q region. Guinier plots here indicate the presence of particles of 600–800 Å. A second linear region at higher Q in the Guinier plot corresponding to a mean particle size of about 200 Å develops with time until it dominates the plots after 100 min, but there is no indication of an interference maximum in the scattering curve. The average particle size achieved is of the order of 500 Å, but the Guinier plots become nonlinear after long growth times, characteristic of a large distribution of particle sizes, or of irregular shaped particles.

If the nucleation time is increased to 20 h at 720 °C there is no induction period, but we observe a linear growth of intensity with time during the first 30 min before the rate declines slightly (Fig. 2). A broad but easily observed interference maximum is detectable after 5 min of growth, now centred at $Q = 0.018$ Å^{-1}, corresponding to an inter-particle spacing of 350 Å or a particle density of $\sim 2\times 10^{16}$ cm^{-3}. The particle sizes are now much smaller, being detectable at 20 Å and growing to only 55 Å after 100 min. The crystallised glass in this case is transparent, whereas non-nucleated and nucleated at 720° for 10 h are respectively white opaque and blue translucent after the crystallisation at 835 °C.

The significance of the results is that they provide information about the state of nucleation immediately before the growth. For the pretreatment of 20 h at 720 °C the immediate growth in SANS shows the existence of a large population of stable nuclei larger than the critical radius R_c. These are particles for which the Gibbs free energy will decrease with increasing size favouring immediate growth once the temperature is raised to give enough thermal energy for diffusion. The immediate presence of a maximum, however, indicates that the nuclei are present in an ordered manner, and gives a measure of the nucleation density. As the initial particle size is considerably smaller than the inter-particle distance, it is unrealistic to consider that the spacial order is due to development of extended excluded regions around the particles caused by depletion of growth material in the glassy matrix or by an Ostwald ripening mechanism[7]. Such mechanisms would only generate an interference peak in the advanced stages of growth of the precipitate, so it is necessary to adopt an alternative hypothesis in which the homogeneous quenched glass undergoes structural changes during nucleation which lead to an enhanced probability that subsequent nucleation proceeds in an ordered or partly ordered manner.

The behaviour after a heat treatment time of 10 h is typical of many of the heat treatments we have performed, where an induction period is observed in the growth kinetics. It is reasonable (after Walton[8]) to consider that this period is composed of three parts

$$t = t_i + t_n + t_g$$

where t_i is the time to obtain a steady state in the cluster distribution, t_n is the time required for nucleation, and t_g is a part of the growth period where the particles are too small to be detected. Thus t_g does not influence the form of the growth curve, but simply makes the early part unobservable and so can be neglected. If we equate t_i with the time to develop concentration fluctuations, and t_n with the time to grow nuclei to the critical size then we can explain our results so far. During 10 h nucleation at 720 °C we develop metastable fluctuations or clusters in the glass, but fail to grow nuclei above R_c. On heating to 835 °C the microstructure is such that nuclei develop rapidly in only 10 min. The nucleation density is, however, much lower than after 20 h at 720 °C as many subcritical nuclei will redissolve at the higher temperature. This is shown by comparison of the size of the particles formed, although in no case have the particles reached the limiting radius. For the glass with no pretreatment we expect a small nucleation density arising from the imperfect quench from high temperatures. The results indeed show rapid growth of a few nuclei which disappear out of the lower end of our Q window, followed by the formation and growth of new nuclei at the end of a long induction period. In this case the induction period will include the time to establish a glass microstructure at 835 °C, followed by growth of the nuclei to the critical size. However, any nuclei present immediately begin to grow, generating a depleted region which limits the development of other nuclei. As we observe, the nucleation density is therefore smaller and disordered, and a wide particle size distribution in the crystals is seen.

These results show the scope of SANS in examining nucleation and growth in glasses. The technique we used, which as far as nuclei are concerned is analogous to a photographic development process, demonstrates that there are important structural changes taking place at temperatures below the glass transition temperature (T_g for this glass is 760 °C). In the cordierite glass system the possibilities for further examination of the time-temperature zone of nucleation are obvious. SANS in our view is the most promising way to explore the effects of the numerous catalysts (such as TiO_2, ZrO_2, and P_2O_5) which are employed in glass ceramic systems. Although in the present experiment we have been aided by the scattering contrast provided by titanium, SANS methods should be generally applicable to the study of nucleation and precipitation in glasses and probably other inorganic systems. Full details of the present work will be described elsewhere.

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The structure of α′-copper phthalocyanine and its susceptibility to radiation damage

CHANGES in electron diffraction patterns are a very sensitive monitor of the radiation damage experienced by a periodic specimen under observation in an electron microscope. In the study of α′-copper phthalocyanine reported here, we have recorded the diffraction pattern of the a^*–c^* plane as irradiation proceeds, and from its behaviour we have been able to confirm the structure of the undamaged crystal and put realistic limits on the electron dose allowable if molecular images of a given resolution are to be obtained. We can also confirm the suggestion of Isaacson *et al.*[1] that the heavily damaged material forms a regular matrix, and that this matrix has approximately the same structure as that observed in previously published molecular images of this specimen[2,3].

The electron diffraction pattern produced by a crystalline specimen comprises a regular array of spots, the intensity of each being proportional to the square of the amplitude of the electron wave function at that point in the diffraction plane. If, somehow, the correct phase can then be ascribed to each amplitude, the wave function is completely determined and its form in any other plane may be calculated. In particular, the Fourier transform of the diffraction plane wave function may be calculated, and within the validity of the kinematic approximation, this is proportional to the electrostatic potential of a unit cell averaged over all the lattice sites. In the case of a perfect crystal this is identical to the potential of any individual cell, but as irradiation proceeds the system becomes a mixture of damaged and undamaged molecules. In these circumstances, although the Fourier transform of the wave function at the diffraction spot positions is still proportional to the average potential, this quantity need not be identical to the potential of any individual cell. Nevertheless, the concept of the average unit cell of such a mixture is a useful quantity. This is because the electron dose experienced by a radiation-sensitive specimen must be limited if there is to be a high probability that the molecules under observation are undamaged. When recording a high resolution image in these conditions very few electrons contribute to each picture element and so the intensities recorded will be substantially affected by statistical fluctuations. Thus in most studies of molecular images there must be some sort of periodic averaging to recover structure from these noisy intensities, and so the behaviour of the average unit cell with increasing irradiation dose is a valid guide to the molecular detail we can hope to obtain. Furthermore, electron diffraction patterns are less affected by lens aberrations than are electron images, and directly useful diffraction patterns may be recorded with very much smaller irradiation doses. We have therefore used diffraction patterns rather than images to study these crystals from the virtually undamaged state to that where no appreciable long range order remains.

Thin crystals of α′-copper phthalocyanine were produced by epitaxial deposition on a heated (300 °C) KCl substrate and were subsequently covered by a thin evaporated coating of carbon. The carbon film–crystallite combination was then floated off on water. Electron diffraction patterns were obtained in a JEOL JEM 100C electron microscope and recorded on Kodirex crystallographic X-ray film. Subsequent densitometry was carried out on a Joyce Loebl flat bed densitometer, the intensities of each spot in the pattern yielding a quantity proportional to the square of the amplitude of the corresponding Fourier component of potential.

To determine the required diffraction plane wave function we must supply phases to each of these measured amplitudes. We proceeded by noting that the structure of platinum phthalocyanine has been determined and that the resemblance between its X-ray powder photographs and α′-copper phthalocyanine have led to the suggestion that their structures are similar[4]. However, recently published molecular images of α′-copper phthalocyanine have indicated that the molecules, whose shape is not in doubt (Fig. 1*a*), may be rotated with respect to those in platinum phthalocyanine[2,3].

To identify the correct orientation we examined these two possibilities to see which was consistent with our single crystal electron diffraction pattern data. Initially, we considered the structure isomorphous with platinum phthalocyanine and by assuming suitable theoretical potentials for each atom, calculated the theoretical diffraction plane wave function for this model. An experimental wave function was then constructed by combining the measured amplitudes with the calculated phases. If the phases are correct then the Fourier transform of this wave function should yield a structure very like that of the model. The resulting reconstruction (Fig. 1*b*) is indeed very similar to the model, individual atoms being resolved in many instances. When the orientation of the model is rotated through angles as small as 1° and the new phases combined with the measured amplitudes, agreement between model and reconstruction is less, and for rotations $>5°$ parts of the molecule becomes unrecognisable in the reconstruction. Thus we are confident that α′-copper phthalocyanine has the same structure as platinum phthalocyanine and that the molecule is orientated with respect to the lattice as shown in Fig. 1*b*.

As irradiation proceeds individual molecules become damaged and deform. Although we do not expect them to do so in an identical way we would expect that because the molecules were originally centrosymmetric, the average unit cell of a mixture of damaged and undamaged molecules will also be centrosymmetric. As such, the phases attributable to each diffraction spot will still be 0 or π. Furthermore, as irradiation proceeds we would not expect to see a discontinuous change in the phase from 0 to π which would produce a change in the sign of the Fourier component of potential. Rather we would expect either a gradual diminuation of the spot intensity to zero followed by a reappearance of the spot corresponding to a phase change, a gradual variation to a new value without falling to zero implying no phase change, or a gradual diminuation to zero corresponding to loss of all long range order of that periodicity. In our study, although many intensities did vary appreciably, none passed through a zero before reappearing. Thus we believe that the original phases should be used in each of the Fourier reconstructions of the partially damaged crystals. Using this procedure we have recovered the average electrostatic potential after various electron irradiation doses (Fig. 1*c*, 1*d*).

As we can see, the behaviour is quite consistent with an increasing number of molecules becoming seriously disrupted, losing long range order of high resolution detail, but retaining some order at low resolution. The structure observed in (Fig. 1*d*) is strikingly similar to previously published[2,3] molecular images and provides experimental confirmation that the technique is valid. In the diffraction pattern corresponding to this reconstruction, all but the first three orders of spots have completely faded and the remaining intensities are relatively stable. We conclude that this averaged unit cell corresponds to the radiation-resistant matrix of damaged molecules suggested by Isaacson[1], where although seriously disrupted, the material retains some periodic detail.

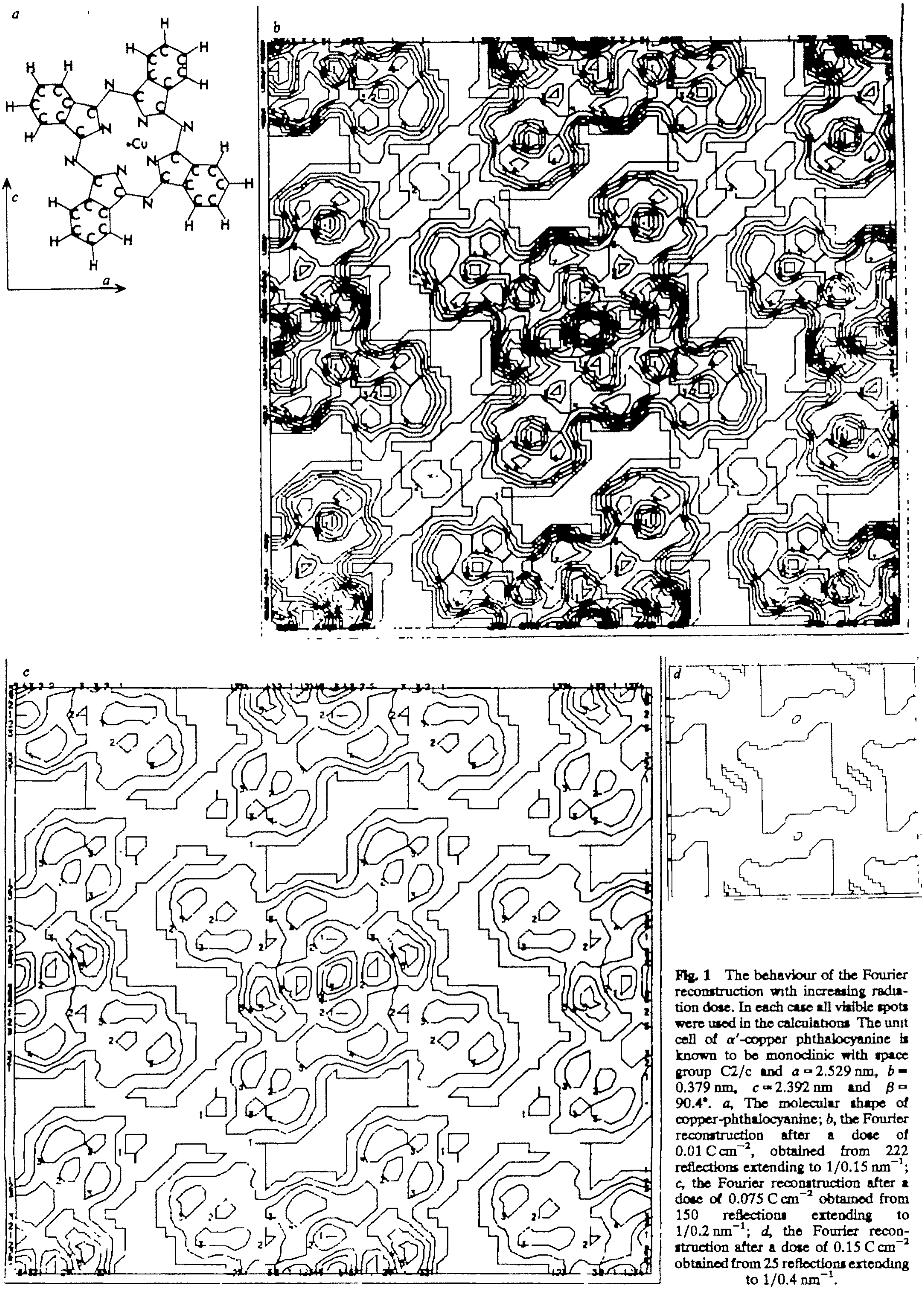

Fig. 1 The behaviour of the Fourier reconstruction with increasing radiation dose. In each case all visible spots were used in the calculations The unit cell of α'-copper phthalocyanine is known to be monoclinic with space group C2/c and $a = 2.529$ nm, $b = 0.379$ nm, $c = 2.392$ nm and $\beta = 90.4°$. *a*, The molecular shape of copper-phthalocyanine; *b*, the Fourier reconstruction after a dose of 0.01 C cm^{-2}, obtained from 222 reflections extending to 1/0.15 nm^{-1}; *c*, the Fourier reconstruction after a dose of 0.075 C cm^{-2} obtained from 150 reflections extending to 1/0.2 nm^{-1}; *d*, the Fourier reconstruction after a dose of 0.15 C cm^{-2} obtained from 25 reflections extending to 1/0.4 nm^{-1}.

From the behaviour of these reconstructions we suggest that for high resolution detail to be preserved the irradiation dose should be $<0.01\ \mathrm{C\ cm^{-2}}$ and that the presence of periodic detail in images of this and similar materials recorded with greater values correspond increasingly to damaged material.

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Radiation pressure and 'aether drag' in a dispersive medium

ARNAUD[1] has pointed out that there should be no transverse 'aether drag' in an artificial dielectric consisting of parallel plane conducting elements, where the product of the phase and group velocities of electromagnetic waves should be equal to the square of the velocity of light *in vacuo*. This agreed with the formula derived by Player[2] and Rogers[3], who for some important examples of aether drag showed that the Fresnel drag coefficient $(1-1/n^2)$, where n is the refractive index, should more precisely be replaced by $(1-1/n_\phi n_g)$, where n_ϕ and n_g are respectively the (phase, or normal) refractive index and the 'group' refractive index.

The same factor $(1-1/n_\phi n_g)$ seems to arise in the division of momentum into what may be described as 'electromagnetic' and 'mechanical' components when a packet of radiation enters a dispersive medium[4]. If the momentum of the packet *in vacuo* is p, the total momentum associated with the packet in the medium is $n_\phi p$, of which a portion p/n_g corresponds to the 'electromagnetic' momentum and $n_\phi p(1-1/n_\phi n_g)$ to mechanical momentum bodily in the medium. Thus, for any medium in which $n_\phi n_g = 1$ as in a wave guide or artificial dielectric, the mechanical component of momentum is zero, and the total momentum should be entirely electromagnetic.

The foregoing conclusion was derived by a thought-experiment involving a modified Einstein light-box to which were applied the relationship $E = mc^2$ and the consideration that actions inside a closed system cannot change the position of its centre of mass. Ginzburg and Ugarov[5] have come to the same conclusion in a more detailed consideration of a particular case, where they conclude: "it is curious that for an isotropic (dispersive) plasma with $n^2 = 1-(\omega_0^2/\omega^2)$, the equation G^M (the electromagnetic momentum) $= G^A$ (the total momentum) holds." For it is also true that for such a plasma $n_\phi n_g = 1$.

The Ginzburg and Ugarov conclusion applies to a medium where the electrons are free, and not when they are bound as in a dispersive non-conducting solid. In that case some of the wave energy is carried as potential energy of electron displacement, and there is a consequent sharing of momentum. With free charges there is no corresponding potential energy of mechanical displacement from fixed centres and so it is at least plausible that there is no mechanical momentum drawn from the wave momentum. A common factor in the artificial dielectric and the plasma is the presence of free (inside the conducting elements) rather than bound electrons.

A corollary of the absence of mechanical momentum when $n_\phi n_g = 1$ seems to be that any medium satisfying this condition will also exert no 'aether drag'. A moving plasma in free space should therefore conform to this expectation, a conclusion also reached by a more detailed argument for the particular case of a cold plasma by Ko and Chuang[6].

A moving plasma can exert an aether drag, in apparent contradiction to the foregoing conclusion, when the plasma consists of moving carriers inside a semiconductor moving parallel to the direction of the light, as observed by Moss, Burrell and Hetherington[7]. I thank M. A. Player for resolving this contradiction—the solution lies in the fact that the plasma is now embedded in a medium of refractive index different from unity, with its faces in motion relative to the frame of reference in which the plasma is stationary. Such a situation, as pointed out by Einstein[8] and Landsberg[9], gives a modified Doppler shift, and when this is allowed for, a result consistent with Moss's observation is obtained.

An interesting variation of Moss's experiment would be to look for an aether drag effect when the free carriers in a semiconductor are moving transversely to the direction of the radiation.

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Plasma convection in force-free magnetic fields as a mechanism for chemical separation in cosmical plasmas

DURING the past two decades, progress in space research has forced us to abandon the earlier idea of a cosmical plasma as being a homogeneous medium. Filament formation and chemical separation are two important phenomena that are often observed in cosmical plasmas and illustrate the importance of inhomogeneity. An average composition from the Sun's atmosphere used to be taken as a standard when expressing cosmic abundancies in general. Numerous observations, however, of the chemical composition in the solar wind from *in situ* measurements and from lunar soil and meteorite samples, indicate a great variability in these ratios[1]. The proton to α-particle flux ratio for solar flare energetic particle events varies widely between and within events[2]. Recent observations show that this variability is connected with the flare process itself and the earlier history of the flare, rather than propagation effects between the flare site and the observer. Some local chemical differentiation mechanism in the preflare history of the specific active region is probably responsible for the composition of the emitted, energetic particles. The phenomena of filamentation and chemical separation may be coupled, as in the presence of a temperature gradient, the plasma convection associated with filamentary structures provides an effective means of selective transport. The general principles of this mechanism are described here. These principles may be important not only in solar flares but in many other cosmical plasmas where force-free fields and thermal gradients occur.

Typical filamentary structures, such as prominences and solar flares, can often be pictured as helical twisted magnetic flux tubes[3–6], where the magnetic field is approximately force free. The electric current flows almost in the direction of the magnetic field.

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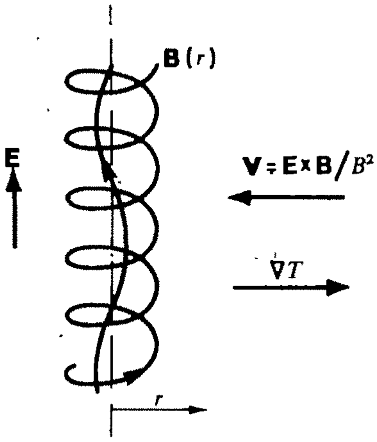

Fig. 1 General form of the magnetic field line pattern in a force-free axisymmetric filamentary structure. The ionised components of the plasma are convected inwards with a velocity V across a temperature gradient ∇T.

Consider for simplicity an axisymmetric case with a force-free helical magnetic field **B**, and a homogeneous electric field **E** applied parallel to the axis of the filament, to drive the current (Fig. 1). We assume that we have a transparent filament where the temperature decreases towards the axis of the filament due to a preferential cooling by radiation of the densest regions.

Under the influence of electric and magnetic fields the ionised component of the plasma will drift inward from the surroundings towards the centre of the filament with a velocity $\mathbf{V} = \mathbf{E} \times \mathbf{B}/B^2$, directed radially inwards. Some steady state models of filamentary structures, where this inward drift of plasma is balanced by an outward diffusion of neutrals, have been described elsewhere[7].

This $\mathbf{E} \times \mathbf{B}/B^2$ convection is a very efficient process for collecting material to form the filament. This is true, even if the process is slowed by collisions, because as long as the particles are charged they are forced to drift inwards.

If the plasma is partially ionised—as in part of the solar atmosphere and many other cosmical plasmas—a temperature gradient will cause the radial transport to be different for elements with different ionisation potentials.

The most abundant elements of a cosmical plasma can be divided into groups of roughly equal ionisation potentials as follows: element, (approximate ionisation potential): He, (24 eV); H,O,N, (13 eV); C,S, (11 eV); Fe,Si,Mg, (8 eV).

Consider, for example, a case where the ambient plasma consists of hydrogen and helium in some initial proportion $p_0 = \{n(\mathrm{H}) + n(\mathrm{H}^+)\}/\{n(\mathrm{H_e}) + n(\mathrm{H_e^+}) + n(\mathrm{H_e^{2+}})\}$ where $n(\ )$ denotes the density of the element within the parentheses. In the configuration considered the ionised components of the plasma will drift inwards and experience a temperature decreasing with radius. When the charged particles have drifted so far that the local temperature is well below the ionisation potential of helium, the rate of recombination of ionised helium is considerable. The neutral helium produced is unaffected by the electric and magnetic forces that drive the ionised particles inwards. On the other hand, the hydrogen is still almost fully ionised, and is therefore still under the influence of the inward force. The protons will continue to drift inwards until they reach a radius where the temperature is well below the ionisation potential of hydrogen. Thus helium and hydrogen will be preferentially deposited in different regions (which may partly overlap). Compared with the composition of the initial ambient plasma, the above process will result in an enrichment of hydrogen in the inner parts ($p > p_0$) and of helium in the outer parts ($p < p_0$).

Although this discussion has considered a very simple configuration, the process works under very general circumstances. Any $\mathbf{E} \times \mathbf{B}/B^2$ convection in a multicomponent plasma will tend to separate elements of different ionisation potentials whenever spatial temperature gradients cause the different elements to leave (or join) the convection preferentially in different regions of space. Note also that the $\mathbf{E} \times \mathbf{B}/B^2$ convection can be a very rapid transport process compared with diffusion. (The latter will in general tend to counteract the separation. However, in thermally inhomogeneous, partly ionised plasmas, even diffusive processes can lead to non-homogeneous chemical distributions[8-10].)

In the case of the flare process, the separation mechanism discussed above might be of importance for the great variability in composition observed. Because an equilibrium composition requires some time to get established in this way, an individual flare or the first flare in a series of flares will show a different composition of particles than that of a successive flare, which shows a composition characteristic of the partially re-established equilibrium. However, here the main purpose is to emphasise the existence of a separation mechanism intimately linked to force-free filamentary structure, and to describe the basic operative principles and potentially wide applicability.

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Persistence of rainfall anomalies in the central Pacific

THE behaviour of the atmosphere and ocean in the equatorial Pacific region, involving the 'Southern Oscillation' and '*El Nino*' phenomena, has been the subject of many studies[1-5]. Attention has been drawn to the notable persistence of anomalies for a year or more, the spatial correlation of sea surface temperature (SST), rainfall and pressure over large areas, and the strong correlation between atmospheric and oceanic features. Two types of mechanism may explain these phenomena and why they are so marked in that region: (1) thermal inertia, for example, some region of the ocean has a large capacity for heat storage, and the heat takes many months to build up or become depleted; (2) positive feedback, for example, if stronger winds in some region favour colder water, and colder water favours stronger

Table 1 Stations used in rainfall index

Station	Lat	Long	Period of data
Nauru	1° S	167° E	1941, 1945–71
Ocean I.	1° S	170° E	1941, 1947–71
Canton I.	3° S	172° W	1942–67, 1972–78
Fanning I.	4° N	159° W	1941–78
Christmas I.	2° N	157° W	1941–77
Tarawa	1° N	173° E	1948–78

Table 2 For each month: *a*, standard deviation of rainfall index *I*; *b*, regression coefficient of *I* on *I* for the previous month

Month	*a*	*b*
Jan	45	0.88
Feb	35	0.64
Mar	28	0.59
Apr	20	0.49
May	27	1.04
Jun	25	0.60
Jul	28	0.83
Aug	28	0.57
Sep	38	1.00
Oct	45	1.04
Nov	51	0.98
Dec	44	0.75

Data for 1941–78.

winds, then even if the thermal inertias of both features are such that their time constants are a month or less, anomalies can persist for many months[6]. New evidence is given here to support a feedback explanation.

Monthly rainfalls at the stations listed in Table 1 were obtained. A preliminary eigenvector analysis indicated that seasonal rainfalls at all these stations vary coherently. The monthly values were transformed by calculating cube-roots, to reduce the otherwise dominant influence of the wettest months on the analyses. Using the transformed data, each individual monthly value was then expressed as a percentage of the mean for that month over the datum period 1948–67. Finally, an index, *I*, was calculated for the period 1941–69 as the mean over stations 1–5, and for 1970–78 (when complete data for stations 1 and 2 were not available) as the mean over stations 3–6. Thus *I* is a series from which the annual variation has been removed, and in which no time smoothing has been performed.

Part of the series *I* is shown in Fig. 1*a*. Its persistence is remarkable, especially by comparison with almost any other meteorological series. Lag correlations for 1–6 months are, respectively, 0.79, 0.69, 0.56, 0.42, 0.31 and 0.20.

If the data are stratified by months, some interesting properties are evident. Table 2*a* shows that the variability of the index is least in April and then increases fairly steadily to 2.5 times its April value by November. Figure 2 shows that month-to-month persistence is quite strong throughout the year, and very strong during the period September–February. Also, there is notably strong persistence over several months from September to February, and weaker but still substantial persistence from June to the following March. Persistence is much weaker during March to June. The breakdown occurs at the season of lowest variability; the greatest persistence occurs in the months of highest variability. These facts indicate that anomalies that develop initially around May to August tend not only to persist, but even to amplify. The amplifying property is emphasised by the regression coefficients of each month's index on the previous month (Table 2*b*), which are greater than unity in some months. Thus the regional rainfall has the following remarkable property: given the September anomaly, the best estimate of the October anomaly is an anomaly of the same sign and more extreme.

The pattern of negative lag correlation on the right side of Fig. 2 indicates a slight tendency for the anomaly to reverse in sign from one September–December to the next, and also a tendency for the sign in January–March to be reversed in the June–December of the following year.

In a typical year the rainfall anomaly in the region changes as follows. In April the anomaly is, in percentage terms, not very great. During May to August, either a dry or a wet regime tends to become established, with a steadily increasing tendency to persist. The regime during July and August is then likely to persist for many months, often until the following March. In particular, the regime in September is very likely to persist at least until January. If August is wet (dry), the next three months are not only likely to continue wet (dry), but also are as likely as not to be wetter (drier). From November to March the absolute value of the anomaly tends to decrease, and the regime shows an increasing tendency to break down. A new regime then tends to develop during April to August. The new regime is more likely than not to be the opposite of that prevailing in the previous year, and there is a hint that in some years, the new regime starts to show itself by January.

Table 3 As Table 2, but for 40 'years' of data generated by feedback model: *a*, standard deviation, *b*, regression coefficient

Month	*a*	*b*
(1)	31	0.42
(2)	28	0.10
(3)	24	0.16
(4)	21	0.31
(5)	29	0.67
(6)	26	0.41
(7)	34	0.70
(8)	43	0.91
(9)	56	1.11
(10)	51	0.79
(11)	44	0.68
(12)	36	0.61

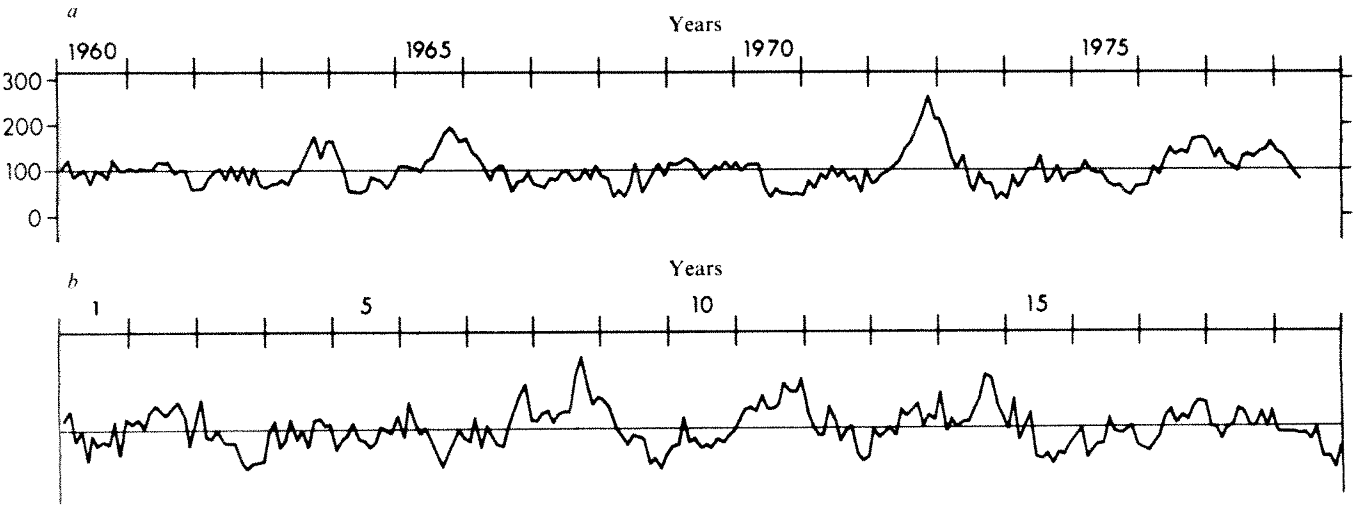
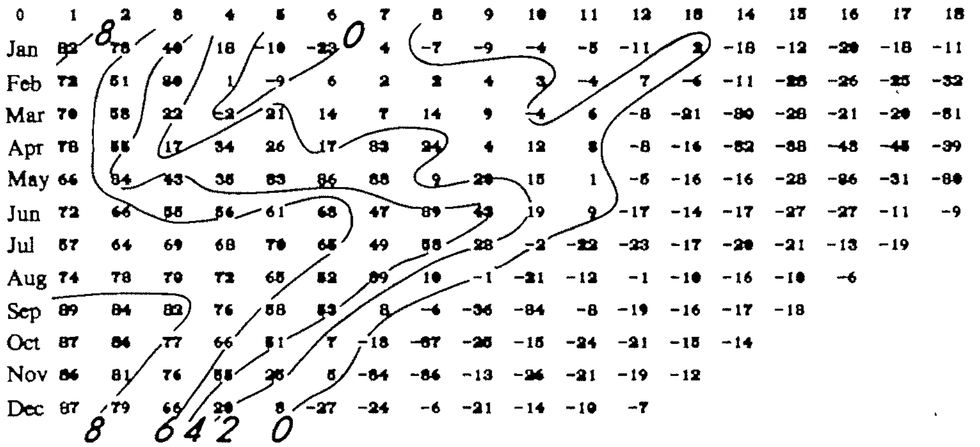


Fig. 1 *a*, Monthly values of rainfall index *I*, 1960–78. *b*, Monthly values of synthetic variable *A* for 19 'years'.



0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Jan	82	73	40	18	-10	-23	4	-7	-9	-4	-5	-11	2	-18	-12	-20	-18	-11
Feb	72	51	80	1	-9	6	2	2	4	3	-4	7	-6	-11	-25	-26	-25	-32
Mar	70	58	22	-2	21	14	7	14	9	-4	6	-8	-21	-80	-28	-21	-20	-81
Apr	78	55	17	34	26	17	83	24	4	12	8	-8	-16	-82	-88	-48	-45	-39
May	66	34	43	36	83	86	88	9	20	15	1	-5	-16	-16	-28	-86	-31	-80
Jun	72	66	56	56	61	68	47	89	43	19	9	-17	-14	-17	-27	-27	-11	-9
Jul	57	64	69	68	70	65	49	58	28	-2	-22	-23	-17	-20	-21	-13	-19	
Aug	74	78	70	72	65	52	89	10	-1	-21	-12	-1	-10	-16	-10	-6		
Sep	89	84	82	76	58	53	8	-6	-36	-84	-8	-19	-16	-17	-18			
Oct	87	84	77	66	51	7	-18	-67	-26	-15	-24	-21	-15	-14				
Nov	86	81	76	58	26	5	-64	-86	-13	-26	-21	-19	-12					
Dec	87	79	66	20	8	-27	-24	-6	-21	-14	-10	-7						

Fig. 2 Lag correlations of I in each month with I in the subsequent months. For example, January with February is 0.82; January with March 0.73; April with the following April −0.08. Data for 1941–78.

These relationships have obvious direct value for statistical prediction. It will be particularly valuable to monitor the development of the anomaly during May to September.

These properties of the rainfall series strongly indicate that some positive feedback mechanism, with the strength of the feedback varying through the year, is operating. To support this statement, I will examine the behaviour of a synthetic variable A involved in a feedback relationship with a second variable B, with external forcing F acting on A, according to the model:

$$\frac{dB}{dt} = -k_A(B-A) \quad \text{and} \quad \frac{dA}{dt} = -k_A(A-qB-F)$$

When F is constant, this system tends to equilibrium if q is <1, and diverges if q is >1. When F comprises a random series, the solutions exhibit fluctuations that are stable if q is <1, and divergent if q is >1, a property noted by Hasselman[7]. Here we consider the system

$$\Delta B = -0.02(B-A)\,\Delta t$$
$$\Delta A = -0.08(A-qB-F)\,\Delta t$$

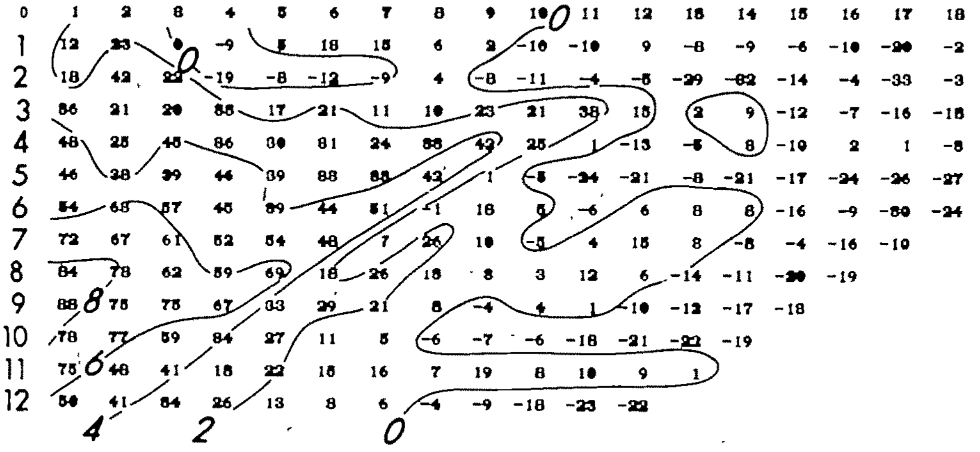
in which the unit of time is taken to be 1/50 month, so the time constant of $A(1/0.08)$ is a week, of B a month. F is the Markov series

$$F_k = 0.8F_{k-1} + E_k$$

where $[E_k]$ is a series of random numbers, so F has a spectrum similar to that of weather fluctuations. In this model A always influences B, while the influence of B on $A(q)$ is given the values 0.4 in months 1–3, 0.8 (4–6), 1.3 (7–9) and 0.8 (10–12).

A sample of A is shown in Fig. 1b, and its statistical behaviour over 40 'yrs' is summarised in Table 3 and Fig. 3. The instability characteristic of months 7–9, when q is >1, takes the form of strong persistence, increasing variability, and regression coefficients near or exceeding 1.0. This behaviour is similar to that of the rainfall series I around October. Moreover, the general characters of the series I and A are similar. Closer similarity could be obtained by making appropriate changes to the constants in the model, but this has not been pursued. This comparison is sufficient to indicate that the behaviour of I can be explained as the result of a positive feedback relationship between two features with time constants of a month or less, in which the feedback is least around April and greatest around October.

For physical justifications, it must be shown that there are features whose mutual influences do have those properties. At present only a qualitative explanation can be offered. Rainfall in the equatorial central Pacific is least when the surface easterly winds there, that is, the Walker circulation, is strongest[1]. Therefore I probably represents an index of the Walker circulation. A stronger Walker circulation favours more upwelling of cold water along the Equator due to Ekman divergence, and also stronger advection of colder water from the south-east Pacific; at the same time, colder SST in the equatorial-east Pacific implies a stronger surface temperature gradient along the Equator, which would favour a stronger Walker circulation[8]. This combination of mechanisms implies positive feedback, which could explain the persistence in I. However, there are other mechanisms of interaction between the Walker circulation and the SST; for example, strong Walker circulation is associated with decreased cloud cover in certain areas, which would favour warming of the ocean. Further, as the Walker circulation interacts with other atmospheric and surface features, additional feedback relationships may be involved. Any or all of these feedbacks might vary through the year; for example, stronger Walker circulation might favour stronger surface winds over the Indian Ocean in one season (favouring surface cooling) and weaker winds in another (favouring warming). All such interactions need to be identified quantitatively before we can claim a complete physical explanation of the statistical properties of I.



0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	12	23	0	-9	5	18	15	6	2	-10	-10	9	-8	-9	-6	-10	-20	-2
2	18	42	22	-19	-8	-12	-9	4	-8	-11	-4	-5	-29	-62	-14	-4	-33	-3
3	86	21	20	88	17	21	11	10	23	21	38	15	2	9	-12	-7	-16	-18
4	48	25	45	86	30	81	24	88	42	25	1	-13	-5	8	-10	2	1	-8
5	46	38	39	44	39	88	83	42	1	-5	-24	-21	-8	-21	-17	-24	-26	-27
6	54	68	57	45	89	44	51	-1	18	5	-6	6	8	8	-16	-9	-50	-24
7	72	67	61	52	54	48	7	26	10	-5	4	15	8	-8	-4	-16	-10	
8	84	78	62	59	69	18	26	18	8	3	12	6	-14	-11	-20	-19		
9	88	75	75	67	33	29	21	8	-4	4	1	-10	-12	-17	-18			
10	78	77	59	84	27	11	5	-6	-7	-6	-18	-21	-22	-19				
11	75	48	41	18	22	18	16	7	19	8	10	9	1					
12	50	41	84	26	13	8	6	-4	-9	-18	-23	-22						

Fig. 3 Lag correlations of A (output of the feedback model) in each month with A in the subsequent months. Data for 40 'years'.

We have shown that rainfall anomalies in the equatorial central Pacific have remarkably high persistence during part of the year. The temporal patterns of lag autocorrelations and variability are readily explainable in terms of a positive feedback relationship in which the strength of the feedback is a function of season. Around October the feedback is so strong that the system displays the potential for 'runaway' behaviour. The feedback probably operates between the Walker circulation (on which the rainfall depends) and the SST, but studies of all the interactions between the Walker circulation and surface features will be necessary to confirm this quantitatively. Prospects are good for successful prediction; the index should be monitored particularly during May to September, because anomalies that develop then have a strong tendency to persist until the following February–March.

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Acoustic attenuation in Lake Tanganyika

THE Lake Tanganyika acoustic attenuation experiment of April 1970 (ref. 1) was the second of two lake experiments designed to help identify the cause of the low-frequency attenuation anomaly in seawater by providing comparative freshwater data. Although the Lake Tanganyika results showed no evidence of the anomaly, the attenuation was unaccountably large. Recent laboratory experiments have shown that the anomaly arises mainly from a chemical relaxation involving boric acid[2]. We have been investigating pertinent chemical equilibria using the acoustic resonator technique[3] and we have encountered another chemical relaxation–absorption in seawater[4] that is evidently also responsible for the Lake Tanganyika results.

Table 1 Chemical relaxation data

	North Atlantic	Lake Tanganyika	Lake Superior
	(concentrations in mg mol l^{-1})		
Mg	53	1.7	0.1
ΣCO_2	2.5	7.2	0.5
Ca	10	0.3	0.3
SO_4	27	0.03	0.03
$B(OH)_3$	0.5	0?	0?
Temp. (°C)	4	23	4
*p*H	8.0	8.5	7.4

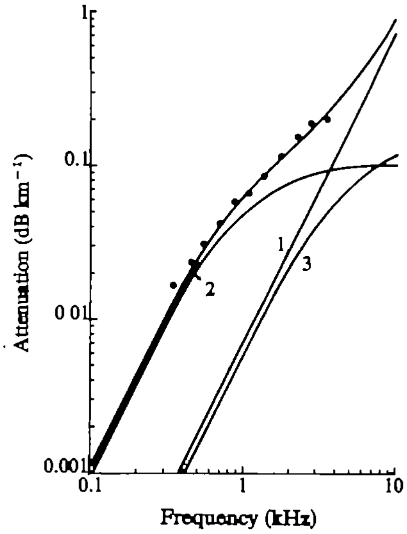

Fig. 1 Three relaxation components of sound absorption in the North Atlantic Ocean: 1, $MgSO_4$; 2, $B(OH)_3$; and 3, Mg/CO_2. The relaxation curves are of the form $\alpha_n = A_n f_n f^2/(f^2+f_n^2)$, where α_n is the component absorption coefficient (dB km^{-1}), A_n is a constant depending on the parameters, f is frequency, and f_n is the relaxation frequency. the top curve is total absorption and points are experimental field data.

Figure 1 shows the relative contributions of the three chemical relaxations in seawater, specifically in the North Atlantic Ocean deep sound channel. The newly discovered third relaxation involves magnesium and carbonic acid with calcium acting to reduce the component absorption by roughly a factor of two. Like that of the boric acid relaxation, the absorption varies as 10^{pH} for pH < 9. (The nominal pH range in the world ocean is

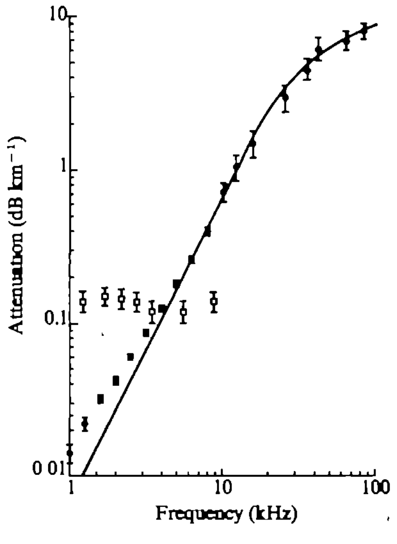

Fig. 2 Comparison of Lake Tanganyika (●) attenuation and synthetic lake water (○) results. The synthetic lake water constituents are $MgCl_2$, $NaHCO_3$ and $CaCl_2$ with appropriate values for Lake Tanganyika from Table 1. The *p*H is adjusted with NaOH. (Na and Cl concentrations are irrelevant.) The solid line is a relaxation curve-fit to the resonator data with relaxation frequency 40 kHz. The excess Tanganyika attenuation at low frequencies is believed to be caused by scattering. Lake Superior (□) also shows a frequency-independent scatter attenuation which is much greater because the water is shallower and the sound channel consequently weaker. Similarity to the $B(OH)_3$ absorption curve of Fig. 1 originally led us to the hypothesis that the seawater anomaly might also be present in freshwater which was, of course, contradicted by the Lake Tanganyika experiment.

7.7–8.3). The relaxation frequency varies from 4.5 to 40 kHz in the temperature range 0–30 °C.

Concentrations of the essential chemical components in the North Atlantic Ocean, Lake Tanganyika, and Lake Superior (the first lake experiment)[5] are given in Table 1 together with temperatures and *p*H values on the respective sound channel axes. Figure 2 shows a comparison of the Lake Tanganyika attenuation and the laboratory resonator results with synthetic lake water. The solid line is a relaxation–absorption curve-fit to the resonator data. The small systematic excess seen in the Tanganyika data at low frequencies is believed to indicate an additional frequency-independent attenuation arising from diffusive scattering by random temperature inhomogeneities within the sound channel[6]. This mechanism is evidently solely responsible for the results of Lake Superior where the scatter loss is greater because of the weaker sound channel and the chemical absorption is negligible[7].

We conclude that the acoustic attenuation in Lake Tanganyika is due primarily to a chemical relaxation–absorption involving magnesium and carbonic acid with a calcium moderator. Although neither the reaction nor even the chemical species involved are yet known, the results may be of wider interest than simply acoustical because of potential chemical and biological implications.

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Glaciation in Bolivia before 3.27 Myr

AN *in situ* layer of ignimbrite overlies till near the base of a thick sequence of glacial and fluvioglacial sediments at La Paz, Bolivia. Two radiogenic age determinantions on the ignimbrite gave results of 3.27 ± 0.14 and 3.28 ± 0.13 Myr. We show here that this indicates that climatic cooling in the Southern Hemisphere was sufficiently severe to produce a mountain ice cap over part of the tropical Andes before 3.27 Myr and that these mountains had probably become tectonically elevated to about their present altitude by that time.

One of the most complete vertical sequences of late Tertiary to Recent strata in Bolivia is in the suburbs of La Paz where streams draining westwards and southwards from the Cordillera Real have cut gorges over 800 m deep into a variety of sediments (Fig. 1). Composed mainly of fluvial and fluvioglacial gravels, sands, silts and clay and till, with minor beds of lacustrine sediments and a layer of volcanic ash flow debris (ignimbrite), the deposits have been studied in most detail by Dobrovolny[1]. Table 1 summarises his interpretation of the sequence, which contains evidence of four major glacial episodes following the deposition of the La Paz Formation, a series of deposits of late Tertiary–early Quaternary age; the ignimbrite was erupted after the first glaciation.

A more recent revision of the area by Servant[2] subdivides the deposits rather differently but retains a similar sequence of four glaciations occurring after the La Paz Formation. One important departure from Dobrovolny's interpretation is the identification of two different beds of ignimbrite, one in the upper part of the La Paz Formation but before the first glaciation and a second resting on deposits of the first glaciation. He identifies these as the Chijini and Sopari ignimbrites respectively but does not describe them in detail; they appear only in his composite diagram of Plio–Quaternary stratigraphy (Fig. 2, on page 324 of ref. 2). Servant's analysis of the latter was based partly on his identification of four denudation surfaces in the north-west part of the Altiplano; he estimates that the oldest formed before the first glacial expansion from the Cordillera Real. A further proposal is that the oldest surface is the same age as an ignimbrite (Formación Peréz[2]) found in the western Altiplano 100–150 km west and south-west of La Paz. As this ignimbrite has an age of 2.5 Myr (Evernden *et al.*[3]) the implication of Servant's work is that the first glaciation occurred after that date. These conclusions are based upon rather tenuous stratigraphical interpolation over large distances, however, and had Servant dated the two ignimbrites appearing in his stratigraphical diagram a more definitive statement could have been made concerning the age of his first glaciation.

I have recently encountered a small but new section in the northern suburbs of La Paz exposing a bed of ignimbrite overlying what appeared to be a layer of till at an altitude of ~3,900 m. The exposure is located in the gorge of a small left-bank tributary of the Rio Choqueyapu or Kaluyo (Fig. 1), the same valley in which Dobrovolny observed his 'Chijini' ignimbrite overlying the oldest till.

The deposit beneath the ignimbrite is a very indurated layer of unsorted clasts up to 30 cm in size held in a compacted clay-like matrix; it averages 2 m in thickness. Out of three samples of 25

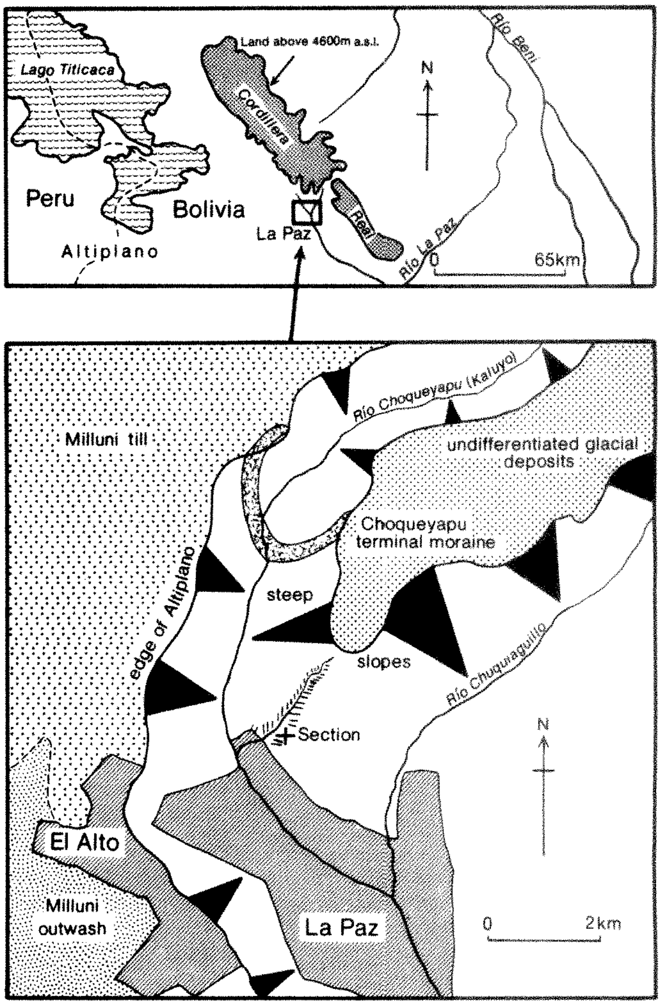

Fig. 1 Maps showing the location of the La Paz area in Bolivia and the site of the new section.

Table 1 Summarised description and interpretation of deposits in the La Paz districts of Bolivia*

	Period	Formation label	Description	Weathering	Maximum thickness (m)
Holocene	Post glacial	—	Landslide, mudflow, alluvium, etc	—	—
	Glacial	Choqueyapu	Fluvioglacial outwash terminal moraines	Little weathered	200
	Interglacial	—	—	Erosion	—
	Glacial	Milluni	Fluvioglacial sediments till layer	Not deeply weathered	80 100
Pleistocene	Interglacial	Purupurani	Alluvial and lacustrine sediments including lignite >32,000 yr old	Intense erosion, plantation; upper 50 m (at least) oxidised	245
	Glacial	Calvario	Till layer	40 m depth oxidised, much eroded away	85
			Fluvioglacial sediments		16
	Interglacial	Chijini	Ignimbrite	Weak erosion intense erosion	15
	Glacial	Patapatani	Till layer	Most intense oxidisation	7
Pliocene			Fluvioglacial sediments		
	Preglacial	LaPaz	Fluvial sediments —upper —middle —lower	Oxidisation Oxidisation	500

* After Dobrovlny

stones, 70% were sub-angular in shape and 30% angular; several were well faceted and bore the polish and striations normally associated with glacial abrasion. More than 50% of the sampled stones were granitic, the rest being mainly hard sedimentary rocks; a few basic igneous stones were also present. Three samples of 25 stones with distinct long axes (ratio >2 : 1) were measured to see if there was a preferred alignment of the clasts. Fifty-six per cent of all stones were orientated within the arc of 120–140°, 92% lying between 120° and 170°; in other words a strongly preferred orientation that could be roughly classified as NW–SE or SE–NW. The former alignment would be entirely in keeping with the direction of ice flow at the base of an ice mass spreading outwards from the adjacent Cordillera Real (Fig. 1). The lower two-thirds of the deposit is a purple-grey colour whereas the upper third has been oxidised to a red-brown colour.

Because these characteristics and the stratigraphical position of this deposit are very similar to those of the lowest till identified by Dobrovolny ~5 km further up-valley, it is interpreted here as the same deposit. Dobrovolny considered this till and associated fluvioglacial sediments to have been much more profoundly oxidised than any of the subsequent overlying Pleistocene deposits and suggested that a considerable length of time elapsed before the eruption of the Chijini ignimbrite.

At the new section a bed of ignimbrite 2–3 m thick rests on the weathered upper surface of the indurated till. This volcanic deposit is a well known phenomenon of the La Paz district, outcropping as a distinctive single layer 2–15 m thick in the main gorges. Dobrovolny observed the ignimbrite above the oldest till in upper reaches of the valley but noted that it directly overlies the preglacial La Paz Formation elsewhere. A sample of the ignimbrite submitted for a radiogenic age determination gave the results shown in Table 2. Two radiogenic ^{40}Ar analyses were performed on a pure biotite sample prepared from the ignimbrite. There is a close agreement between the two determinations, and McIntyre, who did the radiogenic analysis, considered that the measured age of 3.27–3.28 Myr is a good estimate of the volcanic event which produced the deposit.

Table 2 K–AR Analyses of biotite in ignimbrite, La Paz

K (wt %)	Mass (mg)	^{40}Ar* ($\times 10^{-7}$ SCC g^{-1})	^{40}Ar*/^{40}Ar$_T$	Age* (Myr)
7.11	104.9	9.037	0.222	3.27±.14
	91.7	9.070	0.243	3.28±.13

* Decay constants from Steiger and Jager[4].

The main implications of these age determinations concern the age of the La Paz Formation, the approximate timing of the tectonic elevation of the Cordillera Real, and the onset of full glacial conditions in these mountains.

Opinions on the age of the La Paz Formation (which is up to 500 m thick) have varied from Miocene to Quaternary, the uncertainty arising because of the lack of fossiliferous and other datable remains. Dobrovolny, however, after summarising all previous work, concluded that the scant fossiliferous evidence (the vertebrates *Scelidothereum* and *Parahipparion*) obtained from middle beds of the formation indicate a Lower Pleistocene age for these strata whereas the non-fossiliferous and coarser upper beds grade conformably into the first glacial outwash deposits. He tentatively assigned an Upper Pliocene age to sediments composing lower parts of the formation lying beneath a truncated deeply oxidised horizon which separates them from the middle beds. The fossiliferous evidence is so dubious, however, that it cannot be used as compelling evidence for determining the absolute age of the associated strata. If the radiogenic age of 3.27 Myr for the overlying ignimbrite is correct, then the entire sequence of the La Paz Formation is of Pliocene age.

As pebbles of granite from plutonic intrusions in the Cordillera Real are contained in lower beds of the La Paz Formation the mountains must have become tectonically elevated and eroded some time before 3.27 Myr. This agrees with Petersen's[5] view that the latest phase of Andean uplift began in the Pliocene after the formation of the Puna plantation surface in Peru, Bolivia and Chile.

The spatial extent of till sheets (including the earliest) and piedmont moraines along western flanks of the Cordillera Real suggest that a mountain ice cap built up and discharged coalescent outlet glaciers 15 km beyond the foothills during periods of full glaciation. The earliest till layer, overlain by the dated ignimbrite, thus indicates that a substantial mass of glacier ice was present over these mountains more than 3.27 Myr ago. It is tempting to equate this glacial event with the first large-scale glaciation of the southern Andes dated at 3.5 Myr by J. H.

Mercer[6]. Such correlation may be reasonably valid because Dobrovolny observed that the oldest till is intensely oxidised, suggesting a considerable time interval before the deposition of the overlying ignimbrite. Because till of the penultimate ice cap glaciation, exposed for possibly more than 100,000 years, is weathered to only a shallow depth, the intense alteration of the oldest till may well indicate a time interval of the order of 0.23 Myr. The first Bolivian glaciation would therefore most logically fit with the first Patagonian event.

Oxygen isotope and palaeomagnetic evidence from the Northern Hemisphere led Shackleton and Opdyke[7] to suggest that the last lengthy period of stable sea level (global) and low ice volume (global) ended ~3.2 Myr ago. Mercer[8], however, has since drawn attention to his evidence from Patagonia which shows that ice age cold prevailed in middle latitudes of South America 0.3 Myr earlier. This corresponds with recent work in the Barents Sea area of the Northern Hemisphere by Kvasov and Blazhchishin[9] suggesting the existence of an ice sheet there 3.5 Myr ago. The deposits in Bolivia indicate that climatic conditions in the Southern Hemisphere had become sufficiently severe more than 3.27 Myr ago to allow the formation of an ice cap over a mountain range within the tropics. If the altitude of the Cordillera Real (present peaks between 5,000 and 7,000 m) has not been changed substantially by tectonic activity within the past 3.5 Myr, then the equilibrium line of the tropical ice cap was low enough to discharge outlet glaciers down to 3,900 m above present sea level. There is therefore an accumulation of evidence, deriving from polar regions[9,10] and from middle and tropical latitudes of the Southern Hemisphere, which strongly suggests that the cold period of 3.5 Myr ago may have caused the first global large-scale glaciation of the Late Cainozoic ice age.

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A stratified water column environmental model for the type Kimmeridge Clay

GALLOIS has reported the widespread occurrence of coeval coccolith limestones and oil shales in the Kimmeridge Clay of southern England and Lincolnshire[1,2]. From the association of these limestones with oil shale horizons he suggested that the organic content of the Kimmeridge Clay was largely derived from phytoplankton blooms whose decay produced the temporary anaerobic bottom conditions necessary to preserve organic matter. However, comparisons with similar Quaternary sediments in the Black Sea and Mediterranean has led us to report here that phytoplankton blooms were not the cause, but rather a symptom of widespread anaerobic bottom conditions, and that preservational factors rather than productivity are the major control on the accumulation of black shales. We believe that the fine grained clastic-bituminous shale–oil shale–coccolith limestone lithologic association characteristic of the type Kimmeridge Clay can be attributed to the vertical movement of the $O_2:H_2S$ interface in a temporarily stratified water column.

Figure 1 summarises the main features of the oil-shale and coccolith-rich portion of the type succession of the Kimmeridge Clay. The whole sequence was apparently deposited below wave base with only rare and indirect evidence of current activity, such as occasional low angle contacts exhibited by coccolithic streaks within sapropel rich horizons. The Kimmeridge Clay lithologies, with the exception of the early diagenetic dolomites, show a cyclic distribution pattern involving lithologies A–B–C–D (as on Fig. 1). Lithological changes through each cycle are accompanied by changes in faunal content. The clays (A) contain a fairly abundant low diversity macrofauna of small, thin shelled benthonic bivalves, plus a nektonic element including ammonites, fish and reptiles; the plankton includes dinocysts, coccoliths and rare acritarchs. In the bituminous shales (B) the macrofauna is similar to that of the clays, but it tends to occur only at discrete horizons.

In the oil shales and coccolithic limestones (C, D) benthonic fossils are rare or absent, and the non-carbonate organic content consists dominantly of amorphous sapropel with above average proportions of terrigenous palynomorphs[3,4]. High concentrations of Cu, Ni, Mo, P, I and Br[5] are associated with the organic matter. Preliminary scanning electron microscope studies have shown that the coccolithic laminae often contain well preserved coccospheres. Framboidal (probably early diagenetic) pyrite is ubiquitous in the coccolithic limestones, occurring in close association with organic membranes and palynomorphs.

The Quarternary sediments of the Black Sea and eastern Mediterranean basin also demonstrate an association of fine grained detrital, bituminous and coccolith rich lithologies (see Fig. 2 and legend for a summary of their principal lithologies). Radiometric dating of these sequences has enabled them to be matched with the climatic and physical oceanographic data for the Quarternary[7–9], providing an insight into conditions that prevailed at the time of their deposition which we have applied to the Kimmeridgian.

The basal part of the Black Sea sequence (see Fig. 2: Unit Three) contains no marine macrofauna, and its dinocyst assemblage suggests that salinities of about 7‰ prevailed[11]. Unit Two was deposited in marine conditions for it contains marine dinocysts, and marine sapropelic organic matter predominates over terrestrial humic material[10,11]. The organic matter is associated with pyrite framboids and high concentrations of trace elements[12]. The dark laminae within the coccolith oozes of Unit One have recently been interpreted as the products of seasonal mass mortality of the photosynthetic bacteria that live just below the $O_2:H_2S$ interface in the Black Sea[13]. Cooling of the surface waters during the winter decreases the stability of the low salinity surface layer and enables turbulent mixing processes to reach down to the $O_2:H_2S$ interface. The subsequent oxygenation of these lower waters causes mass mortality of the anaerobic bacteria. During the descent of the dead bacteria, the elemental sulphur which they contain probably acts as a nucleus for the formation of pyrite framboids within the water column[13]. Unit Three was deposited in a freshwater environment which was replaced by marine conditions between 10,000 and 7,000 yr ago when the post glacial eustatic rise caused the Mediterranean to spill over the Bosphorus Sill into the Black Sea. This produced a strong salinity-based stratification, and increased the productivity of the surface waters, the combined effects of which resulted in the stagnant bottom waters becoming rapidly anaerobic leading to the deposition of Unit Two. The $O_2:H_2S$ interface rose rapidly from the sea floor until about 3,000 yr ago[14], (see Fig. 2) when it reached a level where convective or turbulent mixing processes could provide its nutrients to the euphotic zone and hence stimulate the coccolith blooms which produced Unit One.

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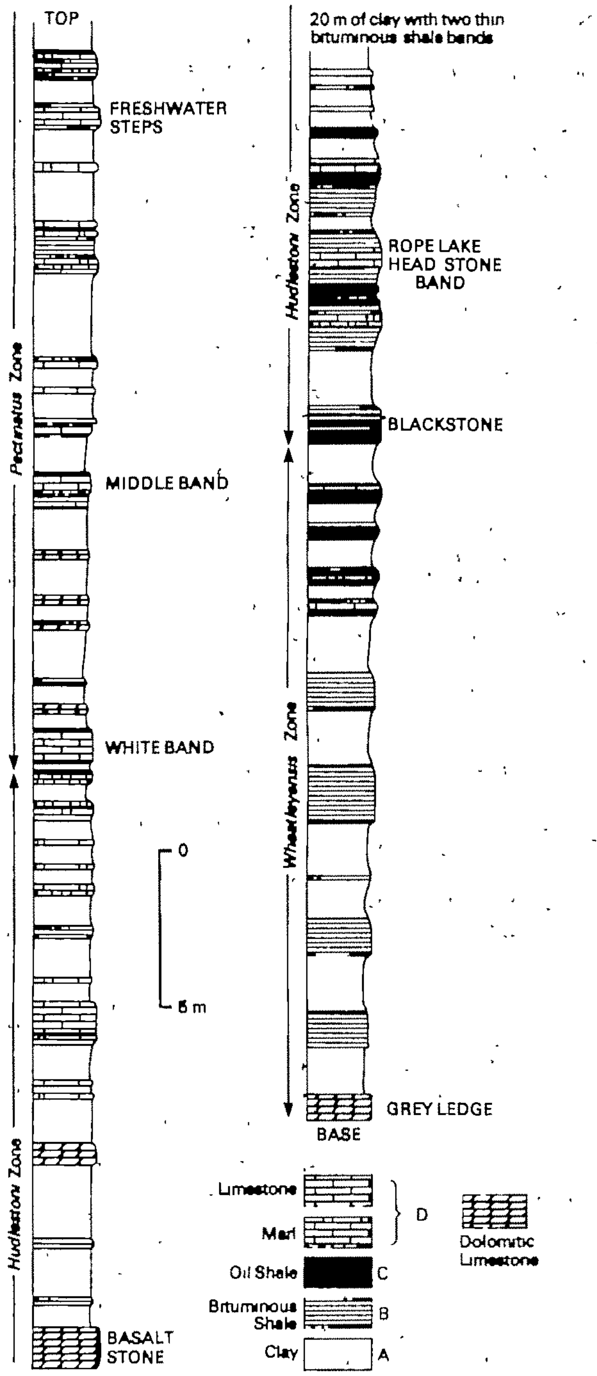

Fig. 1 A log of part of the type section of Kimmeridge Clay exposed in Kimmeridge Bay, Dorset. A, Clays—dominantly composed of illite, with minor silt sized material, up to 10% organic matter (predominantly amorphous sapropel); and ≤2% pyrite. B, Bituminous shales—similar composition to the clays, but higher organic content (≤30%). C, Oil shales—containing up to 70% organic matter. D, Coccolithic limestones—two varieties occur, one homogenised by bioturbation (for example, Rope Lake Head Stone Band), the other showing microlaminations of alternating dark coloured sapropel rich material and light coccolithic layers. Dolomites consist of a microcrystalline mosaic of ferroan dolomite, with amorphous kerogen either as dispersed fragments or discontinuous laminae (~0.25 mm thick). They formed over an extended period from early diagenesis onward by replacement of a pre-existing lithology[6].

The best documented sapropelic sequences of the eastern Mediterranean are those of the Nile Cone[8,16,17], but similar deposits occur throughout the basin[15]. The sapropel sequences are between 30 and 50 cm thick, and are developed between basal terrigenous (turbiditic) sediments and overlying calcareous hemipelagic sediments; the principal lithological components are shown in Fig. 2. They occur below a present day water depth of 550 m, and are best developed in the distal parts of the Nile Cone. Olausson[18] has developed a widely accepted depositional model for the eastern Mediterranean sapropels. The post-glacial amelioration of climate (which significantly altered the balance between evaporation and precipitation[9,15]) coupled with the effects of freshwater overspill from the Black Sea, resulted in a strong halocline which reduced the efficiency of vertical mixing processes and led to the formation of anaerobic bottom water. The simultaneous transgression also caused large amounts of clastics to be trapped on the widened shelves[8], so that there was minimal clastic dilution as well as enhanced preservation of organic matter.

The Quarternary examples imply that the Kimmeridgian sequence: clay–bituminous shale–oil shale–coccolith limestone may reflect the ascent of the $O_2:H_2S$ interface in a stratified water column. However, it is likely that the bottom water was just oxygen depleted for much of the time and did not always contain free H_2S. There are no indications of abnormal salinity in the type Kimmeridgian succession, and so dissolved oxygen was probably a more critical factor in determining the nature of faunal assemblages. The low diversity, thin shelled benthonic bivalve assemblage containing large numbers of small individuals, and the large planktonic component of the fauna are characteristic of low dissolved oxygen situations (~1.0 ml per litre O_2, ref. 19). The small, thin-shelled, dominantly shallow infaunal bivalves are also characteristic of soft 'soupy' mud substrates, their morphology representing an adaptation to flotation within the sediment[20].

Figure 2 summarises the environment of deposition we envisage for each of the main Kimmeridge Clay sediment types. The clays were deposited under oxygenated bottom conditions below normal wave base, partly by hemipelagic settling and partly from low density suspensions. As 75% of the bivalves are articulated, any currents must have been slight, but the occurrence of infaunal species, apparently on bedding planes (as seen also in the Oxford Clay[21]) suggests that at least some exhumation by gentle currents occurred. The bituminous shales formed during periods of fluctuating aerobic/anaerobic conditions with a consequent preservation of greater amounts of organic matter, and the restriction of macro-benthos to discrete horizons. The oil shales were formed in predominantly anaerobic bottom conditions with only very rare current activity and minimal clastic sedimentation. They mark an intermediate phase in the upward progression of the $O_2:H_2S$ interface. The higher proportions of miospores in the oil shales[3,4] (also seen in the Quaternary sapropels[22]) and the sparsity of dinocysts probably indicate a low level of plankton productivity. The coccolith limestones mark the maximum development of anaerobic bottom water in the Kimmeridgian water column. At this time the top of the H_2S zone had migrated to a level where seasonal convective processes could provide its nutrients to the euphotic zone and there stimulate a coccolith bloom. In the upper part of the *Hudlestoni* Zone and the lower part of the *Pectinatus* Zone (see Fig. 1) there are many cycles in which the oil shale is missing but the coccolith limestone is present. Interpretation in terms of our model suggests a shallower depth of water than that lower in the sequence (where there are relatively thick oil shales), because in this case, once the bottom became anaerobic the $O_2:H_2S$ interface would take less time to rise to the thermocline and conditions suitable for oil shale formation would have been short lived. In the modern Black Sea, the most characteristic micro-laminated coccolith oozes occur in the halistatic parts of the basin where there is only a weak annual bloom (in spring, associated with seasonal convection[23]) and minimal clastic

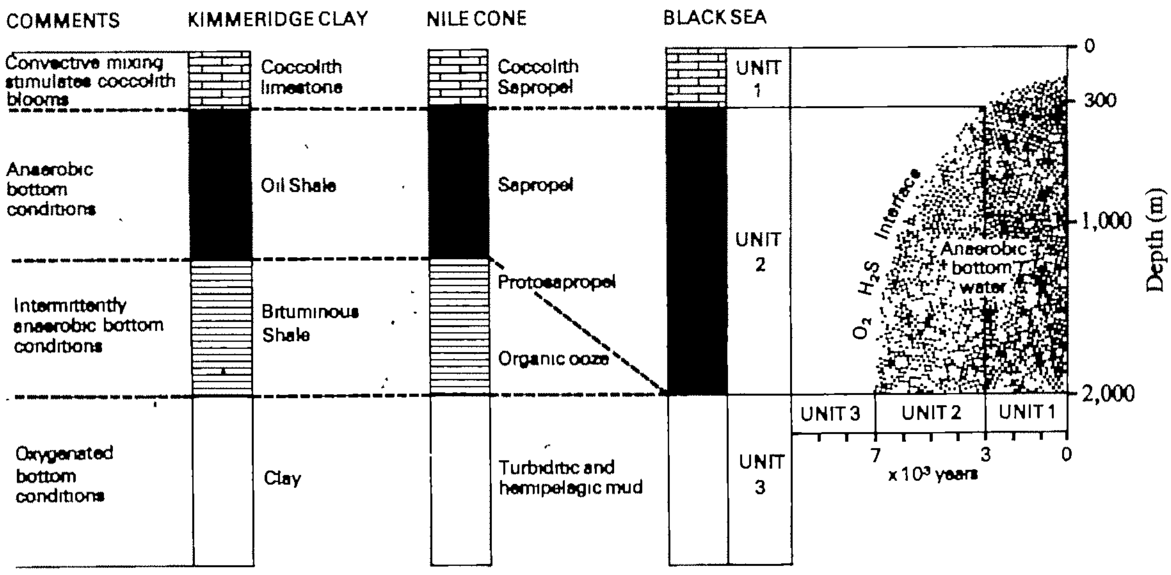

Fig. 2 Lithologies and environmental interpretations for the Kimmeridge Clay and Quarternary sequences discussed in the text. The sapropelic and coccolithic sequences of the Nile Cone and Kimmeridge Clay are interpreted in terms of the upward migration of the $O_2:H_2S$ interface documented in the Black Sea[14], culminating in convectional mixing of surface waters which stimulates coccolith blooms. Kimmeridge Clay lithologies are described in Fig. 1 and in the text. Nile Cone lithologies[8,16,17]: organic ooze—with up to 15% sand grade biogenic material; protosapropel—bioturbated sediment transitional between organic ooze and sapropel; sapropel—black, organic rich ($\leq$15%) sandy mud, including planktonic forams, authigenic pyrite. Some sapropels contain interlaminated coccolithic and terrigenous muds. Benthonic organisms are absent. Black Sea lithologies[7]: Unit One, biogenic calcareous ooze, with light coloured coccolithic laminae alternating with dark organic rich laminae of detrital clays; Unit Two, micro-laminated dark brown gelatinous sapropelic clay with $\leq$25% organic matter; Unit Three, partly homogenous and partly laminated light and dark banded lutites with interbedded turbiditic silts. Column sections are not to scale.

sedimentation. We believe that in this respect the Kimmeridgian is directly analogous. The preservation of the delicate laminations and coccospheres indicates that deposition was entirely by hemipelagic settling and that the bottom remained predominantly anaerobic during the deposition of the limestones. Dunn also believed, on the basis of pyrite distribution, that the coccolith limestones may have been deposited during the acme of anaerobic conditions[24].

Although the Kimmeridgian early diagenetic dolomites do not form part of the cyclothems, their development may be indirectly related to phases of watermass stratification. In the Mediterranean, precipitation of authigenic high Mg–calcites near the sediment surface shows an inverse relationship to the development of watermass stratification[25]. This pattern is also reflected in the Kimmeridgian succession, for dolomites tend to be more common where the oil shales and coccolith limestones are poorly developed (that is, below the *Wheatleyensis* Zone and above the *Hudlestoni* Zone).

The Quarternary examples used as the basis for our Kimmeridgian depositional model represent deep water oceanic environments, whereas the Kimmeridge Clay was deposited in a shallower shelf regime: we submit that this apparent paradox is not a serious challenge to our model. For example, below the $O_2:H_2S$ interface in the Black Sea the environment is remarkably uniform and it is doubtful if the bottom conditions would be greatly different whether it was 2,000 m deep, or just a few hundred metres deep. Thus, although our model cannot add anything to earlier estimates of the palaeo-bathymetry of the type Kimmeridge Clay, it does suggest that the deepest water conditions occurred during the deposition of that part of the sequence where oil shales are most abundant (the top of the *Wheatleyensis* Zone and the bottom of the *Hudlestoni* Zone).

The development of widespread stagnant conditions in North-west Europe during the Kimmeridgian was influenced by both palaeogeography and a rise in sea level. The Lower Kimmeridgian probably marked the maximum extent of the Jurassic shelf areas[26], and the increase in depth of the water column must have separated the mixed surface waters from the sea bottom, creating a stagnant, potentially anaerobic layer. At present, even relatively deep shelf seas are maintained in an oxygenated condition by wind driven currents and tidal mixing, and also by lateral circulation from the oceans. However, in comparison with its modern counterparts, the Kimmeridgian shelf sea was much wider, and the length of its oceanic (Tethyan) margin was small. Moreover, this margin was flanked by a carbonate platform in southern Europe which may have restricted the lateral shelfwards circulation of Tethyan water. The oxygenation of the internal shelf sea may, therefore, have depended largely on (seasonal?) convection and turbulent mixing in the surface waters, with the corresponding tendency for stagnation below wave base.

We can only speculate on what exactly controlled the cyclic ascent and decline of the $O_2:H_2S$ interface. Although in the Kimmeridgian the lithological response to stratification was almost identical to that in the Quarternary, it is clear that quite different mechanisms and controls were operative. The interpretation of the Quarternary examples revolves around the extreme climatic changes that occurred at the end of the last ice age, with glacial meltwaters and the land-locked nature of the basins playing a key role in the depositional model. Such an explanation is clearly incompatible with both Kimmeridgian palaeogeography and the more equable, sub-tropical climate of the period. Given this situation it seems probable that thermoclines, rather than haloclines, were the principal agents of water column stratification and stagnation in the Kimmeridgian. Quite small changes in local rates of subsidence, primary production, circulation pattern and climate may have combined to disrupt the fine balance between aerobic and anaerobic conditions to produce the short term cycles ($\geq$14,000 yr)[5] seen in the type Kimmeridge Clay.

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Reduction of radiation damage in an electron microscope with a superconducting lens system

OBSERVATION of biological specimens at high resolution by electron microscopy is limited by radiation damage. In general, structural details of non-periodical arrangements can only be identified for doses of radiation up to 10^{-3} C cm^{-2}, corresponding to 63 e nm^{-2}, which restricts the resolution to approximately 2 nm (refs 1, 2). Many methods have been applied in an attempt to reduce radiation damage, including high voltage, high vacuum and specimen pretreatment. The most successful, however, was the reduction of the radiation dose, used for certain specimens with periodical structure, where the information could be obtained by computer-aided image enhancement from underexposed films[3]. In special cases, although it was possible to obtain information at high resolution, the specimen was partly destroyed[4]. The advantages of low temperatures were also investigated, but due to the lack of an adequate instrument, no agreement between the results could be obtained. Furthermore, little attention was paid to the significance of the dose rate at low temperatures. We now have in operation an electron microscope with a superconducting lens system in which the specimen is cooled to liquid helium temperature[5]. The most important features of this instrument are the high resolution, which allows the transfer of space frequencies corresponding to the inverse of 0.15 nm, the mechanical and electromagnetic stability (specimen drift 0.01 nm min^{-1}) and the ultra high vacuum around the specimen. These features are necessary for testing the effect of radiation damage. We report here experiments which demonstrate that radiation damage is considerably reduced at low temperatures in a dose rate-dependent manner.

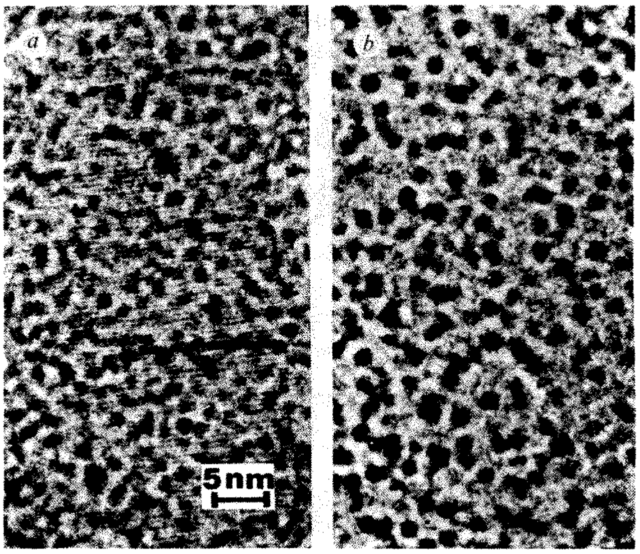

Fig. 1 Pt-stained murein or peptidoglycan, electron-optical magnification × 84,000. For *a* and *b*, respectively, exposure time was 110 and 3 s, dose rate was 3×10^2 and 10^4 e nm^{-2} s^{-1}, total dose during radiation was 3×10^5 and 1.5×10^6 e nm^{-2}, and estimated rise in temperature was <1K and $\geqslant 10$ K. The phase structures in *a* and *b* are not completely identical due to a defocus difference of about 10 nm.

One of the specimens used was murein or peptidoglycan (the rigid layer of a bacterial cell wall), specifically stained with Pt. According to a model published by Formanek[6], 0.45, 1 and 2 nm periodicities in the heavy-atom pattern of murein should be visible. The support was in most cases graphitic oxide, which shows very little phase contrast. In spite of extensive work, this structure has not been seen previously in the electron microscope.

Figure 1*a*, taken with a dose rate of 3×10^2 e nm^{-2} s^{-1} (0.005 A cm^{-2}) clearly demonstrates, even after a total irradiation dose of 3×10^5 e nm^{-2} (5 C cm^{-2}), the presence of 0.45-nm periodicities, which disappear as soon as the dose rate is increased to 10^4 e nm^{-2} s^{-1}(0.2 A cm^{-2}) (Fig. 1*b*). Light optical diffractograms of Fig. 1*a* show reflections corresponding to periodicities of both 0.45 nm and 1.0 nm. Such reflections were also detected on carrier-free murein.

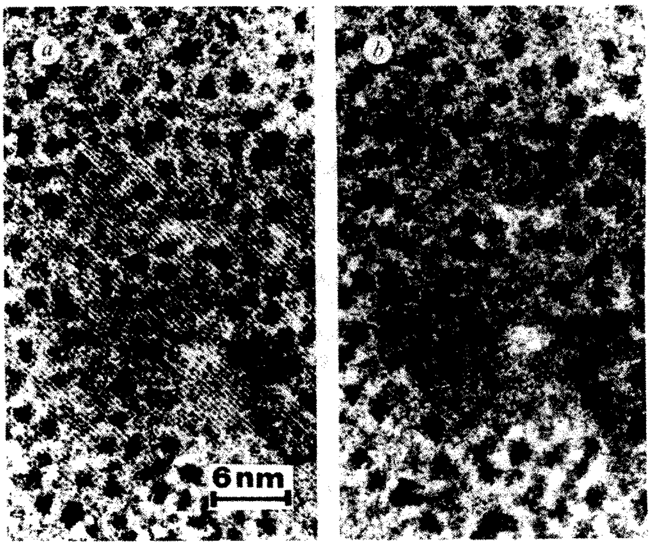

Fig. 2 Pt-stained polyhydroxybutyric acid, electron-optical magnification × 84,000. For *a* and *b*, respectively, exposure time was 120 and 16 s, dose rate was 3×10^2 and 5×10^3 e nm^{-2} s^{-1}, total radiation dose was 1.4×10^6 and 2.5×10^8 e nm^{-2}, and the estimated rise in temperature was < 1 K and $\geqslant 10$ K.

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We also investigated a Pt-stained by-product of the murein preparation. Periodicities were clearly visible as long as the dose rate was kept below a certain critical value (Fig. 2*a*) and faded away when the dose rate was increased (Fig. 2*b*). We have assumed that the material is poly-β-hydroxybutyric acid, as this is the only possible crystalline organic contamination originating from the bacterial cell. In addition, a periodicity of 0.36 nm agrees with one of the main lattice distances of poly-β-hydroxybutyrate[7].

The observations that the conservation of the fine structure of the biological material strongly depends on the radiation dose rate we consider to be due to a temperature effect. The influence of the dose rate on the specimen temperature can be estimated[8]. For the compounds under discussion the chemical bonds are destroyed during the first exposure; however, the diffusion of the molecular fragments and especially the heavy atoms is prevented if the specimen is kept at a sufficiently low temperature. At the critical dose rate, the temperature is increased above the threshold value that permits diffusion. Comparison with the results obtained at room temperature shows that when specimens are observed close to liquid helium temperatures, damage to their biological fine structure is dramatically reduced.

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Recent archaeological findings at the Sterkfontein site

UNTIL the present decade it was thought that the Early Stone Age of Africa consisted of two industrial complexes, the Oldowan and the Acheulian, the latter succeeding the former after a transitional phase. Well excavated and well dated Oldowan sites are known only from Eastern Africa, although Oldowan sites are reported from North and South Africa, and all known examples are older than 1.5 Myr (refs 1–4). The earliest manifestations of the Acheulian appear approximately 1.5 Myr BP (refs 5, 6) and continue until the Last Interglacial. Leakey has challenged the idea that the Acheulian represents a continuous development of the Oldowan in East Africa[1,7,8]. At Olduvai in Bed II she recognises two industrial complexes, which are said to represent two distinct cultural traditions, possibly produced by two different hominid taxa. These industries are the Acheulian and the Developed Oldowan. The criteria for defining the Developed Oldowan B have recently been revised and Leakey[7] now states that the morphology and technical features of manufacture of bifaces are the principal distinguishing characters between the two industries. The main technological distinction is that the Acheulian biface makers knew how to detach large flakes (>10 cm) while the Developed Oldowan hominids did not. As a result of this revised definition some of the assemblages at Olduvai originally defined as Developed Oldowan B have been reclassified as Acheulian by Leakey[7]. The problem of the Acheulian and Developed Oldowan can be stated as two alternative hypotheses. (1) Two cultural traditions began in East Africa approximately 1.5 Myr BP, each associated with a distinct social population over an immense span of time; possibly they were of different hominid taxa. Or (2), there was only one cultural tradition (industry), the Acheulian Industrial Complex, beginning about 1.5 Myr BP and marked by highly variable bifacial tools and relative frequency of artefact types; only one hominid taxon during any given period was involved. I have now made a comparative study of the Sterkfontein and Olduvai Gorge Bed II assemblages, which suggests that the Developed Oldowan B industry falls within the Acheulian Industrial Complex and therefore serious consideration should be given to dropping the former term.

To test the hypotheses four assemblages were selected for comparative analysis from Bed II at Olduvai Gorge, two defined Early Acheulian, EF-HR and TK Lower Floor (TKLF), and two defined Developed Oldowan B, TK Upper Floor (TKUF) and FC West Floor (FCWF). These assemblages were chosen because each is made up of material excavated from a single occupation floor, and therefore each represents a chronologically homogenous set of artefacts presumably produced by one social group of hominids. The other Bed II assemblages do not satisfy this criterion. Table 1 presents the shaped tool type percentages of the four assemblages, demonstrating clearly that the original criteria of the Developed Oldowan are no longer valid. TKLF, an Acheulian assemblage, has a high percentage of spheroids/subspheroids, the most varied light-duty tool component, and a low percentage of bifaces. This assemblage is considered Acheulian by Leakey[7] because of the technique of manufacture of the bifaces. The conclusions of the comparative study will be restricted to bifaces, the taxonomically critical type. Bifaces from the Sterkfontein site are shown in Fig. 1.

Nine attributes out of a larger set were selected to describe the morphological and technical features of the bifaces. The results are presented in detail elsewhere[9,10], including analysis of other artefact types. The application of non-parametric statistical tests (χ^2 and Mann–Whitney U test), measures of the coefficient of

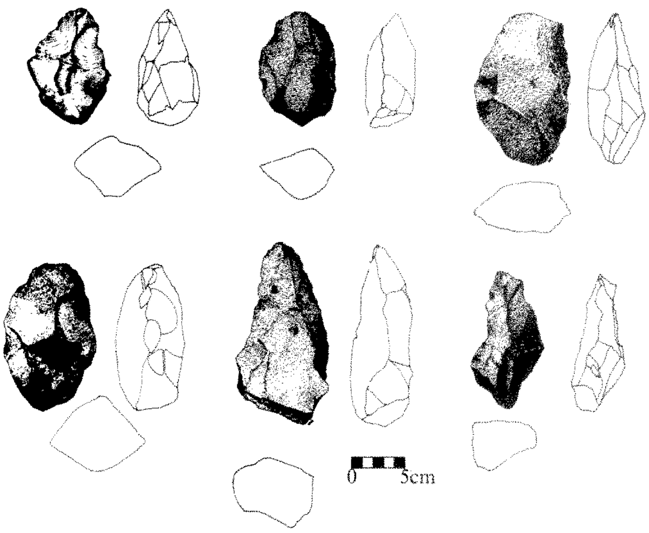

Fig. 1 Sterkfontein bifaces. *a*, Handaxe from cavity infilling of Robinson excavation; *b*, handaxe from overburden; *c*, cleaver from overburden; *d*, handaxe from overburden; *e*, handaxe from overburden; *f*, handaxe from Dump 1. Illustrations by Zoë Martin.

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variation, and computer assisted multivariate programs (average-linkage cluster analysis and discriminant analysis) were made of the five assemblages using the attribute values of the bifaces and resulted in the following conclusions. (1) The bifaces of the defined Acheulian assemblages were significantly different from those defined as Developed Oldowan B; (2) the Acheulian bifaces were longer, thicker, and had longer working edges; (3) the Acheulian bifaces were made significantly more often on large flakes (>10 cm); (4) the differences observed between the bifaces of the two industries were due to the difference in frequency of use of large flakes as the primary form for biface manufacture; and (5) the Acheulian bifaces display an equal degree of standardisation within assemblages as the Developed Oldowan B bifaces.

Table 1 Shaped tool frequencies of four Olduvai Gorge Bed II assemblages

	Site							
	EF-HR		TKLF		TKUF		FCWF	
	No.	%	No	%	No	%	No.	%
Choppers	14	17.7	6	5 1	29	10.2	49	25.9
Proto-bifaces	0	0	0	0	0	0	0	0
Bifaces	37	46.8	9	8.0	18	6 3	4	2.1
Polyhedrons	5	6.3	1	0 9	3	1.0	4	2.1
Discoids	8	10.1	0	0	9	3.2	4	2.1
Heavy-duty scrapers	3	3.8	6	5.1	13	4.6	11	5.8
Spheroids/ subspheroids	9	11.4	28	23.9	76	26.7	48	25 4
Modified battered cobbles, nodules and blocks	0	0	17	14 7	35	12 3	54	28 6
Light-duty scrapers	3	3.8	24	20 5	78	27.4	9	4 8
Burins	0	0	12	10.3	6	2.1	1	0.5
Awls	0	0	8	6 8	15	5.3	2	1.1
Outils écaillés	0	0	2	1 7	0	0	0	0
Laterally trimmed flakes	0	0	4	3.4	3	1.0	0	0
Sundry tools	0	0	0	0	0	0	3	1.6
Totals·	79	99.9	117	100.4	285	100.1	189	100.0

χ^2 Tests of the unmodified whole flakes demonstrated that large flakes in general were present significantly more often in the Acheulian assemblages. Because large flakes were present in the Developed Oldowan B assemblages, Leakey's assertion that culture tradition explained the inability of the Developed Oldowan B peoples to produce large flakes could not be immediately accepted. The alternative explanation of differential use of raw material was investigated.

Use of χ^2 tests showed that large flakes were produced significantly less often from quartz than from lava. Bifaces made from flakes and unmodified whole flakes were of quartz significantly more often in the Developed Oldowan B than in the Acheulian assemblages. These findings suggested that the basic difference between the Acheulian and Developed Oldowan B bifaces was the differential use of quartz and lava as raw material. In the absence of any other archaeological evidence, I suggest that the latter hypothesis be accepted—that is, that the Acheulian Industrial Complex, beginning about 1.5 Myr BP, was marked by highly variable bifaces and per cent frequency of artefact types. The term Developed Oldowan B should be dropped unless archaeological evidence can be produced to establish its independence from the Acheulian.

Using the BMDP 2M average-linkage cluster program on the CDC 6400 computer at the University of California, Berkeley, I found that the Sterkfontein bifaces clustered exclusively with the Acheulian bifaces of EF-HR, but that when assigned as ungrouped cases to the Olduvai assemblages in the discriminant analysis (SPSS program with Mahalanobis D^2 as the distance measure) they were more often grouped within the Developed Oldowan B of TKUF. The Sterkfontein bifaces were also found to be more highly standardised as assessed by the coefficients of variation of biface attributes and the discriminant analysis results[9,10]. A comparison of univariate frequency distributions of attributes of several different artefact types showed Sterkfontein to display no consistent patterns of similarity or dissimilarity with any particular assemblage of the Olduvai industries, but rather it presented an overall pattern of distinctive variability. I conclude that the Sterkfontein assemblage was Early Acheulian and that it displays characteristics that fall well within the range of those of the Acheulian complex of East Africa.

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Jackal helpers and pup survival

BLACKBACKED jackals (*Canis mesomelas*) are one of the few mammalian species to have long-term pair bonds and to exhibit a tendency for some offspring to help in the provisioning and guarding of subsequent litters. Bonded pairs of adults were studied for 28 months over 3½ years at Lake Ndutu, Serengeti Plain, Tanzania. In the 15 litters observed, families with helpers had significantly higher offspring survivorship.

The blackbacked jackal is a small omnivore that typically occurs in brushland habitat[1–4]. Bonded pairs are territorial and both the female and the male mark and defend boundaries. Bonded pairs groom each other, hunt cooperatively[3], and share food. Parental care of pups is approximately equal. Both of them feed and guard the pups and the male regurgitates to his mate during the period of lactation. In all observed boundary fights the aggression occurred between same sex individuals. The territories form a mosaic with core territories in the centre of the *Acacia* sp. and *Balanites* sp. woodland surrounding Lake Ndutu, and peripheral territories adjacent to the short grasslands. Three of four pairs of blackbacked jackals identified in July 1974 still occupied approximately the same territories in February 1978. In August 1976 an additional five pairs were identified.

Eleven out of 15 litters observed had non-parent adult helpers. In 1976–77, five families were observed during two consecutive litters. In 1977, four of these families had helpers and the 'helpers' were pups from the previous year's litter. Thus with monogamous parents the helpers were full siblings to the litter of pups that they were caring for. Given this genealogy, the degrees of relatedness are as follows: parents to offspring, $r = 1/2$; helpers to full sibs, $r = 1/2$; parents to grandpups, $r = 1/4–1/2$ (dependent on degree of inbreeding); helpers to offspring, $r = 1/2$.

Presence of helpers may be partially dependent on the age of the parents. A pair with its first litter would not have any helpers. However, the survival of pups does not ensure the presence of helpers the next year. In 1976–77, four families had 12 surviving offspring. Of these, four stayed as helpers (1 per family). In the fifth family, four pups survived and none remained as helpers.

Helpers contribute food directly to the pups, and also regurgitate to the mother during the period of lactation. The percentage of observed regurgitations by helpers to pups varied from 18 to 32 within families. Helpers also contributed by guarding the pups while the parents were absent. Spotted hyenas do attempt

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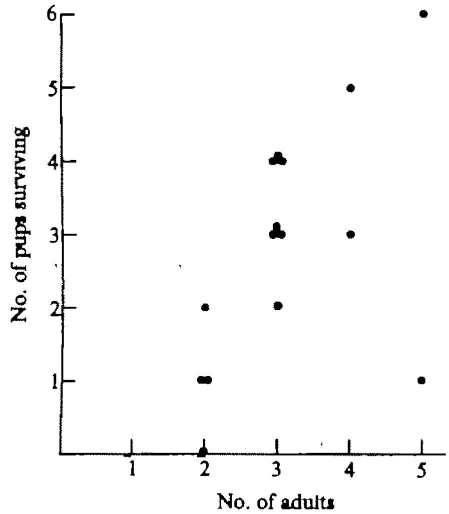

Fig. 1 Number of adults per family and pup survivorship. $n = 15$; Spearman rank correlation coefficient, $R_s = 0.967$; $P < 0.01$.

to prey on young pups, but can be successfully driven away by one adult jackal. Families with more adults (that is, those with helpers) had an attendant adult at the den a higher proportion of the time, thus providing greater protection for the pups. Helpers also play, groom, and help teach the pups to hunt. Quantitative details of helper contributions will be published elsewhere.

Pups first emerge from the den at approximately 3 weeks of age and are weaned at 8–9 weeks. By 14 weeks they are well coordinated, no longer use a den, and are starting to forage with adults. All observed mortality has occurred within this initial 14-week period. This is the time within which the pups are most vulnerable to food deprivation and predation, and the time period in which major contributions are made by helpers and parents.

The presence of helpers correlates positively with pup survivorship and was significant to the 0.01 level (Fig. 1). On average, each helper added 1.5 surviving pups to the litter. One aberrant group (litter number 15) had three helpers initially and only one surviving pup. This is the only family in which a helper has been observed to remain for more than one year, and the parents were old. At 14 weeks of age, only one pup remained, and was cared for almost exclusively by the second year female helper. The other two helpers had disappeared. Thus this family had old parents, and for part of the critical period for pups, only one helper.

The presence of helpers seems to be independent of food density. In the study area, jackal territories in the core and periphery of the woodland vegetation differ in the density of an important prey species, the grass rat (*Arvicanthus niloticus*). In a core territory (VIII), trap–recapture data on *Arvicanthus* density varied from 7,792 (November 1976) to 32,083 rats km^{-2} (July 1977). In a peripheral territory (VII), *Arvicanthus* varied from 3,125 (November 1976) to 13,125 (July 1977) (R. Senzota, personal communication). The ratio of rat density between core and peripheral territories was 2.5:1. However, this difference in food availability did not seem to affect the presence of helpers and pup survivorship. In territory VIII, a core territory with high prey density, the resident pair with one helper had four pups survive from the 1976 litter. None of these pups stayed as helpers. In June 1977, the same pair had a litter of five pups, and none of them survived. By contrast, in 1976, in peripheral territory VII, the resident pair with no helper had a litter of five pups and one survived. This pup remained with his parents. In July 1977, the same pair had a litter of six pups and one helper. Three pups survived. Even in the peripheral area with the lower *Arvicanthus* density, there is still a large biomass available at whelping time ($13,125 \times 60$ g = approximately 800 kg km^{-2}). Thus even in the peripheral area there is probably an adequate food supply and the critical factor in pup survival is the number of provisioning adults that can capture that prey and make it available to the pups.

Hamilton's theory of inclusive fitness seems particularly relevant to the observed behaviour of helpers[5]. Inclusive fitness consists of: (1) the individual's personal fitness in terms of reproductive success; and (2) the effects of his behaviour on the reproductive fitness of his conspecifics multiplied by their degree of relatedness. 'Inclusive fitness', therefore, examines the lifetime effect of an individual's behaviour on the next generation's gene pool, not only in terms of his own reproduction but also that of related individuals[6]. According to Hamilton an individual's behaviour will be favoured by natural selection if it increases inclusive fitness.

Jackal helpers are as closely related to their full siblings as they would be to their own offspring. As their contributions as helpers increase pup survivorship, their own inclusive fitness is enhanced by kin selection until such time that breeding is attempted. In fact, an adult helper gains more (yield 1 pup per adult) by being a helper of its parents than by finding a mate and raising its own pups aided only by its mate (yield 1/2 pup per adult). Helpers may also derive benefits from extended experience on the home territory in terms of increased survivorship, increased long-term reproductive success and acquisition of a portion of the home territory[7]. Parents benefit from the presence of helpers from increased reproductive success due to higher pup survivorship, and increased inclusive fitness as parents accrue more reproductive benefits by helpers investing in their pups ($r = 1/2$) than by helpers producing their own pups (grandpups $r = 1/4$). Thus, it is in the parent's best interest to retain helpers. The benefits of retaining helpers are potentially limited by available food resources.

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Is intragenic recombination a factor in the maintenance of genetic variation in natural populations?

INTRAGENIC recombination between two different existing alleles in a population can create new alleles. The role of this process in maintaining variation in a natural population has been investigated[1] by assuming that a gene consists of two sites, each of which can mutate to an infinite number of unique 'alleles' (the infinite allele model of Kimura and Crow[2]). Using this model it was shown that if the product of the population size, N, and the mutation rate, μ, is $\gg 1$, and the recombination rate, r, is the same order of magnitude as the mutation rate, then intragenic recombination significantly increases the number of alleles maintained in a finite population. Moreover, this is not equivalent to an increase in the mutation rate as the variance of homozygosity and the variance of the number of alleles are larger. This implies that the sampling theory of neutral alleles developed by Ewens[3] does not apply to the situation where intragenic recombination is important in maintaining variation[4]. Application of these results to the study of intragenic recombination in natural populations would require a large number of independent samples in order to estimate the variance of homozygosity and the variance of the number of alleles. However, data from natural populations usually consist of the number of alleles and their frequencies in a single sample. Therefore, for the effect of intragenic recombination to be observable, there must be a detectable change in the distribution of the allele frequencies in a sample which contains k alleles. We present here the results of Monte Carlo simulation which show that intragenic recombination in the equilibrium population increases the frequency of the most common allele, increases the number of alleles which occur only once in the sample, and increases the homozygosity.

The Monte Carlo simulation utilises the same algorithm we used previously[1]. Briefly, for each of the $2N$ genes of the next generation two genes are selected at random with replacement from the present generation; one of the four alleles, the two recombinants and the two non-recombinants, are then selected with probabilities $r/2$, $r/2$, $(1-r)/2$ and $(1-r)/2$, respectively; and lastly, it is determined whether a mutation occurs at each of the two sites with probability ν where $\mu = 2\nu$. This is equivalent to the model of random drift with two loci[5]. For the simulation of intragenic recombination, the parameter values, $2N = 200$, $4N\mu = 4$, and $r = 10\mu$, were used because it has been shown that there is a significant effect of recombination on the allelic variation maintained in a population with these parameter values[1]. A sufficient number of samples, each with k alleles, is obtained in the following manner: each sample is the first generation which contains k alleles and which occurs at least $2N$ generations after the previous sample. Samples are collected at least $2N$ generations apart to minimise the effects of co-variance among samples. The results presented are for 400 samples with $k = 25$ and are representative of those obtained for other values of k. For comparison, 400 samples with $k = 25$ alleles are collected in the same manner with the parameter values, $2N = 200$, $4N\mu = 8$, and $r = 0$. $4N\mu = 8$ is a valid choice of the parameter value for the case of no recombination, as Ewens[3] has shown that the distribution of allele frequencies conditional on k is independent of the value of $N\mu$. The parameter value, $4N\mu = 4$, is not used because the probability that a generation contains 25 alleles is very small.

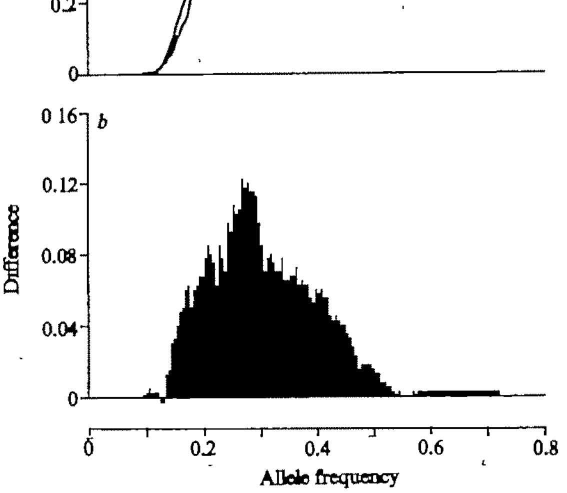

Fig. 1 Cumulative distributions of the frequency of the most common allele from Monte Carlo simulation of intragenic recombination between two sites. For each set of experiments 400 samples were collected, each sample with $k = 25$ alleles, at least $2N = 200$ generations apart. In the case of intragenic recombination, $4N\mu = 4$ and $r = 10\mu$; in the case of no recombination, $4N\mu = 8$. *a*, Cumulative distributions of the frequency of the most common allele; *b*, difference between the cumulative distributions.

Table 1 Statistics of the allelic distributions of 400 samples from Monte Carlo simulation of intragenic recombination

	$r = 0$		$r = 10\mu$	
	Mean	Variance	Mean	Variance
Frequency of the most common allele	0.234	0.00532	0.258	0.00841
Number of singleton alleles	7.255	4.85	7.78	5.63
Homozygosity	0.119	0.00132	0.134	0.00264

$2N = 200$ and $k = 25$.

Three variables are used to compare the allelic distributions of the two sets of samples: the frequency of the most common allele, the number of alleles which occur only once in the sample, and the homozygosity. The cumulative distribution of the most common allele in each of the two sets of samples is shown in Fig. 1, and also the difference between the two distributions. It is seen that the frequency of the most common allele is greater in the case of intragenic recombination than that without recombination. As shown in Table 1, this difference holds for the means and variances of the frequency of the most common allele, the number of alleles which occur only once, and the homozygosity.

Is this pattern of differences observable in data from natural populations? We have stated previously[1] that the observed intragenic recombination rate at the *rosy* locus in *Drosophila*[6] (which varies from 1.2×10^{-4} to 7.2×10^{-6}) and the observed number of alleles in samples from natural populations[7,8] are such that intragenic recombination could have a significant role in the maintenance of variation at this locus. The data of Coyne[7] and Singh, Lewontin and Felton[8] have recently been extensively re-analysed by Waterson[9] and it was found that the frequency of the most common allele is significantly too large in each of the pooled samples. There was also significantly greater homozygosity and the singleton alleles were too frequent. The results of the simulation of intragenic recombination presented here are in the same direction as that observed in these data from natural populations. Therefore, intragenic recombination may be responsible for some of the excess of rare and of common alleles observed in samples from natural populations.

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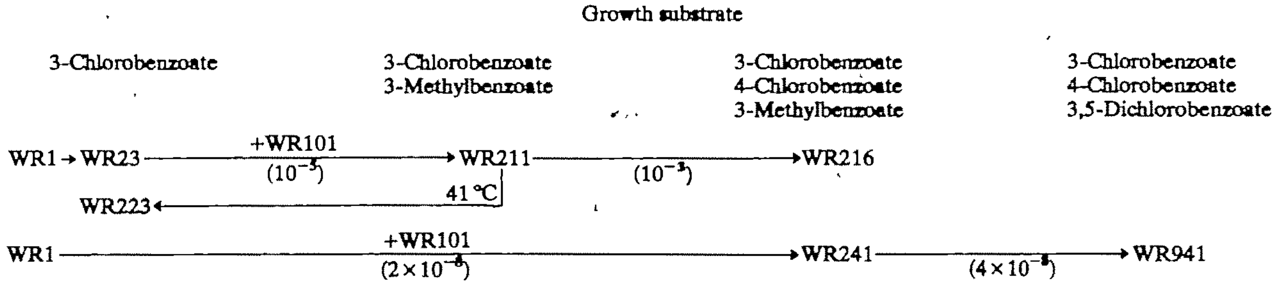
Construction of haloaromatics utilising bacteria

LARGE amounts of novel organic compounds are released into the environment by the rapidly growing chemical industry. The main agents for returning natural and, presumably, synthetic organic compounds to the carbon cycle are bacteria and fungi, provided that catabolic enzymes with appropriate specificities, transport systems and regulatory mechanisms can be activated. One or several of these conditions for total degradation do not seem to be fulfilled for certain types of man-made compounds, in particular, the halogenated aromatic hydrocarbons. In this report, we demonstrate the transfer of the TOL plasmid from *Pseudomonas putida* mt-2 (WR 101) to *Pseudomonas* sp. B13 (WR 1) enabling novel strains to be isolated which can utilise various chlorosubstituted benzoates as their sole source of carbon and energy.

Pseudomonas sp. B13 (WR1) is one of the few bacteria known to be able to use haloaromatics such as 3-chlorobenzoate or 4-chlorophenol as the sole source of carbon and energy[1,2]. Comparative kinetic studies on the 1,2-dioxygenation of benzoate[3], dehydrogenation of dihydrodihydroxybenzoates[4] and 1,2-dioxygenation of catechols[5] indicated that the stereospecificity of the initial enzyme hinders the utilisation of the 2- and 4-chlorobenzoates in addition to 3-chlorobenzoate in this strain. We did, however, demonstrate that the TOL plasmid coded[6,7] benzoate 1,2-dioxygenase in *Ps. putida* mt-2 ((WR101) is considerably less specific and readily co-oxidises chlorosubstituted benzoates, including 4-chlorobenzoate[3]. Nevertheless in contrast to *Ps.* sp. B13, this organism cannot totally degrade halocatechols. Substrate utilisation did not occur, but a number of halosubstituted metabolites of the meta cleavage pathway were accumulated, together with unstoichiometric amounts of chloride (our unpublished work).

Table 1 Construction of halobenzoate-utilising strains



Donor and recipient cultures were grown for 24 h on nutrient agar plates. The bacteria were suspended in phosphate buffer *p*H 7.4, 50 mM to 3×10^8 cells ml^{-1}. Portions of each culture were mixed so that the ratio of donor and recipient was 2 · 1. One ml of the resultant mixture was filtered through 0.2 µm Millipore filter. The filter, after being placed on the surface of nutrient broth agar plates, was incubated for 12 h at 28 °C and then vigorously whirlmixed with 4 ml phosphate buffer to resuspend the cells. The resulting suspension was plated on the appropriate selective medium. The frequency of transfer given in parentheses are defined as the number of exconjugants per donor cell or spontaneous mutation frequency on the selective substrate.

Table 2 Nutritional properties of wild-type and derived strains

Strain designation	Phenotype																			Genotype	Derivation	
	Mcl	Pcl	Dcl	Ptol	Tln	Rib	Seb	41°	Ger	Nic	Pdiol	Eth	But	Pro	Kglu	Man	Test	Hexa	Str		Parent	Treatment
WR1 (*Pseudomonas* sp. B13, wild-type)	+	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	s	wt		Dorn *et al.*[1]
WR23	+	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	r	str-1	WR1	Spontaneous, selection on nutrient agar plates with streptomycin (0.1 mg ml^{-1})
WR101 (*Pseudomonas putida* mt-2, wild-type)	−	−	−	+	+	+	−	−	−	+	+	+	+	+	+	+	−	−	s	wt/TOL		Williams and Murray[6]
WR211	+	−	−	+	−	−	+	+	+	−	−	−	−	−	−	−	+	+	r	str-1/TOL	WR23	Conjugation with WR101, selection on Ptol/Str agar plates
WR216	+	+	−	+	−	−	+	+	+	−	−	−	−	−	−	−	+	+	r	str-1/TOL	WR211	Spontaneous, selection on Pcl agar plates
WR223	+	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	r	str-1	WR211	Spontaneous, during growth at 41 °C on nutrient broth agar plates
WR241	+	+	−	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	s	/TOL-241	WR1	Conjugation with WR101, selection on Pcl agar plates
WR941	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	s	/TOL-241	WR241	Spontaneous, selection on Dcl agar plates

Phenotype abbreviations: Mcl, 3-chlorobenzoate, Pcl, 4-chlorobenzoate; Dcl, 3,5-dichlorobenzoate; Ptol, *p*-toluate; Tln, toluene; Rib, D-ribose; Seb, sebacat; 41°, growth at 41 °C on nutrient broth, Ger, geraniol; Nic, nicotinate; Pdiol, propandiol (1,2); Eth, ethanol; But, *n*-butanol; Pro, n-propanol; Kglu, 2-ketogluconate; Man, D-mannose; Test, testosterone; Hexa, hexadecane; Str, streptomycin sulphate

+, Good growth; −, no growth; s, sensitive; r, resistant.

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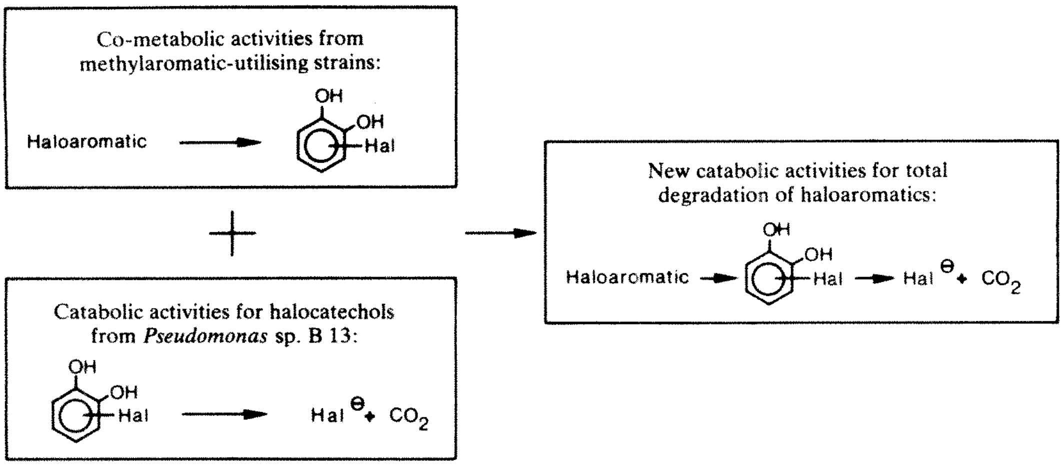
Co-metabolic activities from methylaromatic-utilising strains:
Haloaromatic
OH
OH
Hal
Catabolic activities for halocatechols from *Pseudomonas* sp. B 13:
Hal⊖ + CO2
New catabolic activities for total degradation of haloaromatics:

Fig. 1 Concept for the construction of haloaromatics degrading bacteria from methylaromatic—utilising strains and *Pseudomonas* sp. B13 (Hal = halogen).

Exconjugants like WR 241, which is able to grow on 4-chlorobenzoate (see Table 1) were obtained by the Millipore filter technique after selection on 4-chlorobenzoate agar plates. Differentiation by nutritional pattern (Table 2) and analysis of catabolic enzymes (to be published elsewhere) clearly indicated that WR 241 originates from WR 1 as the recipient strain. The ability to utilise 4-chlorobenzoate is the only new property in strain WR 241. Other exconjugants, not listed here, were isolated which could utilise *p*-toluate in addition to 4-chlorobenzoate. From this, it can be deduced that WR 101 functioned as the donor strain. By mating WR 101 with the streptomycin-resistant strain WR 23 of WR 1 and selection on *p*-toluate–streptomycin agar plates, strains of type WR 211 were found which could use both growth substrates of the parent strains, namely, 3-chlorobenzoate and *p*-toluate. By plating WR 211 on 4-chlorobenzoate, spontaneous mutants like WR 216 were isolated which could grow on the new substrate.

Exconjugants, such as WR 241, appeared with almost the same frequency as the product of the frequencies which were observed during selection for strains WR 211 and WR 216. Phenotypic expression for the utilisation of 4-chlorobenzoate seems to require a mutation in addition to the transfer of the TOL plasmid. During growth at 41 °C strain WR 211 can be cured, consequently, WR 223, the resulting strain, lost its ability to grow on *p*-toluate.

Biological devices for the adaptation to novel organic chemicals are mutations in structural genes leading to the evolution of enzymes with novel specificities and activities[8-11] and mutations changing regulatory controls[12,13]. In another study, the detectable response to stress by novel organic molecules was the hyperproduction of pre-existing enzymes due to gene duplication[14]. The active sites of these enzymes possess a slight affinity for compounds of related chemical structure. The second copy enables mutations to accumulate, which are not immediately constrained by selection. An ideal mechanism for accelerated enzymatic adaption to novel substrates is suggested by the observation that the genes for catabolic enzymes are carried on extrachromosomal elements[15,16].

In the present study, *Ps.* sp. B13 acquired the ability to utilise novel substrates like 4-chloro- and 3,5-dichlorobenzoate by conjugation with *Ps. putida* mt-2. The initial turnover of the halogenated benzoates was provided by the TOL plasmid-coded enzymes, whereas total degradation and utilisation was accomplished by the halocatechol-degrading enzymes of the recipient strain. In addition, exconjugants were observed in which *Ps. putida* mt-2 seemed to have acquired the genes coding for the enzymes of halocatechol catabolism (our unpublished work). In general, the acquisition of the genes of halocatechol catabolism could overcome accumulation of toxic halocatechols which frequently occurs during co-metabolism of haloaromatics in arene or methylarene-utilising bacteria. We found the same enzymes for total degradation of halocatechols in other pseudomonads which are capable of utilising a variety of haloaromatic compounds. These had been isolated from continuous adaptation cultures in the presence of *Ps.* sp. B13. The combination of methylarene-utilising strains providing high co-metabolic activities for halosubstituted analogues by their initial enzymes, together with the halocatechol-degrading enzymes of *Ps.* sp. B13, could be a general concept for the construction of new strains (see Fig. 1) which could degrade halosubstituted benzenes, phenols, anilines and naphthalenes, totally.

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Selection of predation-resistant bacteria in continuous culture

VARIOUS models of predator–prey interactions predict the occurrence of reciprocal oscillations in the densities of the predator and its prey[1]. Some models predict undamped, neutrally stable oscillations (the Lotka–Volterra model), whereas others predict damped oscillations, the two populations gradually approaching an equilibrium. Few attempts have been made to test these models, possibly because of the rarity of suitable experimental systems. The predatory bacterium *Bdellovibrio*, which preys on other bacteria, provides an ideal system in which predator–prey interactions can be studied in a convenient and accurate way. As part of a study of the ecology of *Bdellovibrio*[2] we have inoculated bdellovibrios into a continuous culture of prey bacteria growing in a chemostat. The introduction of the bdellovibrios was followed by reciprocal population oscillations.

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Table 1 Comparison of *Photobacterium leiognathi* E28 (original strain, O) and two strains (A, B) isolated from the chemostat after 6 days of mixed cultivation with *Bdellovibrio*

	O	A	B
Cell morphology	Rod (pairs)	Rod (pairs)	Oval (in chains)
Colony morphology*	Translucent	Translucent	Opaque
Doubling time in batch culture*	22 min	23 min	36 min
Maxima light intensity (quanta s^{-1} ml^{-1}) in batch culture*	6.0×10^{11}	8.8×10^{11}	1.8×10^{9}
Bdellovibrio plating efficiency†	1.0	1.0	$<1.0\times10^{-7}$‡
$t_{1/2}$ (time to reduce prey population to half original size after mixing with *Bdellovibrio* at a ratio of 1:10)	10.3 min	10.3 min	>60 min

* On medium MPY (ref. 3).

† Determined as described by Marbach *et al.*[3].

‡ Macroplaque was obtained when 10^7 or more *Bdellovibrio* plaque-forming units were placed on the lawn.

However, the oscillations did not persist and were not damped. Instead, a new system evolved in which a bdellovibrio-resistant mutant was detected.

The bacterium which served as prey was the luminescent *Photobacterium leiognathi* E28 (ref. 2). It was grown in a chemostat set to hold a carrying capacity of 10^8 cells ml^{-1}. After the culture reached a steady state, a small inoculum of the predator, marine *Bdellovibrio* BM4 (ref. 3), was introduced. Figure 1 depicts the pattern of growth which followed: classical oscillations are apparent up to the sixth day (in this specific experiment); the pattern then changes, as the prey population which was initially homogenous, begins to show morphologically different types of colonies. From this point the prey population consisted of two different genetic types—one resembling the wild type, having a strong bioluminescence (type A), the other being more opaque, and dark (type B). Single colonies of both types were streaked on agar and isolated into axenic cultures. Figure 2 shows the growth curve and the bioluminescence of the two types isolated from the chemostat, in comparison with the strain with which the chemostat was originally inoculated. Type A grows at a similar growth rate and emits light of a similar intensity to that of the original strain; type B grows at a slower rate and emits light at an intensity less than 1% that of the original strain.

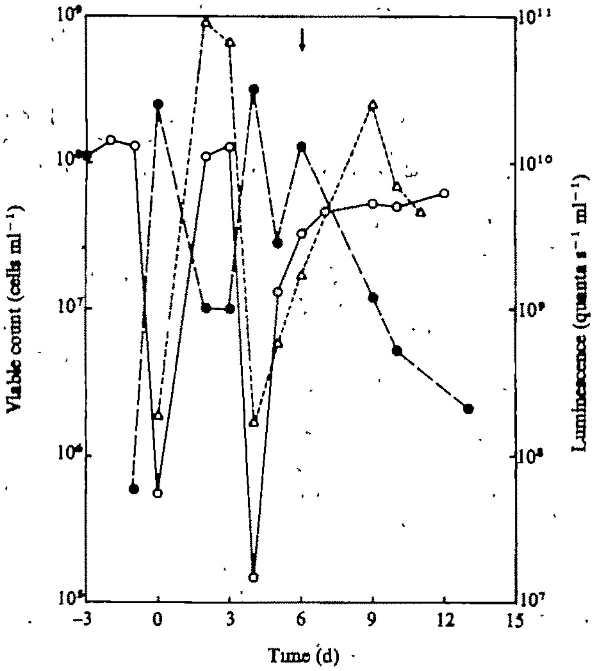

Fig. 1 Growth of *Bdellovibrio* BM4 and *Photobacterium leiognathi* E28 (both strains streptomycin-resistant) in a mixed culture in a chemostat at a dilution rate of 0.1 h^{-1}. The medium used was MPY (ref. 3) diluted 1:15 and supplemented with streptomycin (100 μg ml^{-1}) to reduce the possibility of chance contamination with bacteria. The prey (○) was allowed to reach a steady state, then the flow was stopped and bdellovibrios (●) were inoculated and allowed to grow for 24 h in batch culture. At time zero the flow was turned on again and kept constant throughout the experiment. The triangles indicate the light intensity of the culture, measured by a photometer similar to the one described by Mitchell and Hastings[7]. The arrow indicates the first appearance of mutant colonies on the plates. Viable counts were done as described by Marbach *et al.*[3], using plates supplemented with streptomycin.

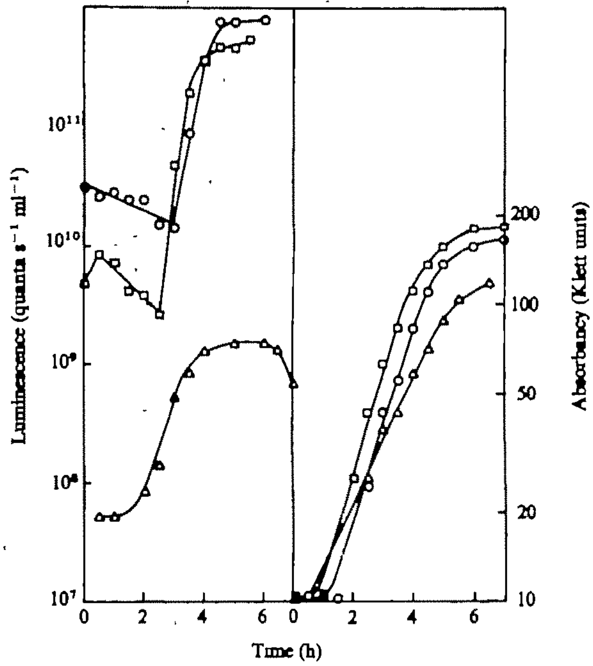

Fig. 2 Growth curve and bioluminescence of *Photobacterium leiognathi* E28 and of the two colony types isolated from the chemostat. □, The original strain inoculated into the chemostat; ○ type A; △, type B. All three cultures were grown in MPY medium in a shaking water bath at 26 °C. Absorbance was measured in a Klett–Summerson photometer using filter no. 42.

When a suspension of each of the two types was mixed with the bdellovibrios (at a ratio of 10 bdellovibrios per prey bacterium), type A cells were immediately attacked and penetrated, as observed under the phase-contrast microscope, while type B cells remained untouched by the bdellovibrios. Death of the type A photobacteria followed the attack by the bdellovibrios (Table 1).

Plaque assay gave a similar picture: the efficiency of plating of the same bdellovibrio suspension on lawns of type B was at least 10^7 times lower than on type A or on the original strain (Table 1).

In summary, a two-membered culture of a *Bdellovibrio* and a fast-growing susceptible prey evolved into a three-membered culture containing bdellovibrios, a prey type similar to the original, and a dark mutant, which grows more slowly and is resistant to the bdellovibrios. The different growth rates of the luminous bacterium and its dark mutant would exclude the possibility of their coexistence in the chemostat unless there is an interaction between the two strains. Indeed, when the two were inoculated together in a chemostat operating in the same conditions as the three-membered culture, but without the bdellovibrios, type A was maintained in a steady state, while type B was washed out. The resistance of the dark mutant to the bdellovibrios would not allow the latter to reproduce so the coexistence of predator and mutant is also unlikely. However, a mutant with slower growth rate and greater predation resistance may be able to coexist with the wild type and its predator in a stable three-membered system. The stability of such a system has been theoretically predicted by Cramer and May[4] who, using simple three-species mathematical models, showed that a two prey–one predator system could be stable in circumstances where the two-prey system would in the absence of predation be unstable due to competition.

The possibility of coexistence of a predator and its prey in a homogenous environment has also been questioned[5]. Van den Ende[6] studying the interaction of a protozoan predator (*Tetrahymena pyriformis*) and its bacterial prey (*Klebsiella aerogenes*) in continuous culture concluded that the coexistence of the two organisms was due to spatial heterogeneity created in the system by the prey bacteria. No such heterogeneity could be observed with the photobacteria, which were homogenously dispersed in the growth vessel; their persistence could be explained by the presence of the slow growing and predation-resistant mutant.

Although *Bdellovibrio* has been known and studied for 16 years, this is the first report of the isolation of bdellovibrio-resistant mutants. Apparently, conditions prevailing in batch cultures do not allow the evolution of such mutants, while growth in a chemostat in the conditions used in this study repeatedly resulted in their appearance.

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Unusual maternal–fetal blood glucose concentrations in Weddell seal

FETAL whole blood glucose levels are usually lower than, and fluctuate with, maternal levels. That is, a favourable concentration gradient is maintained to allow for the facilitated transfer of glucose across the placenta from mother to fetus[1,2]. This situation is apparently reversed in the Weddell seal. The concentration of glucose in maternal whole blood is lower than that in fetal blood during at least three metabolic states: in the resting–fasting state, in simulated diving, and in recovery from diving[3]. We report here that one factor contributing to this unusual relationship between maternal and fetal blood glucose is the relatively low gradient between normal blood levels in fetus and mother. This is made possible by high haematocrit values and a low turnover of glucose in both parent and fetus.

Five pregnant Weddell seals (*Leptonychotes weddelli*) weighing 450–500 kg were captured on the fast ice near Ross Island, Antarctica and taken to the Eklund Biological Laboratory at McMurdo Station. Intermittent blood samples were obtained from indwelling maternal and fetal catheters implanted as previously described[4]. Samples of blood (2 ml) were added directly to perchloric acid and treated as before[4]. Glucose levels in neutralised perchloric acid extracts were determined using the hexokinase assay[5]. In several fetal and maternal extracts, glucose was determined also by the glucose oxidase method, the Benedict assay system and by an automated glucose analyser with a linear response over the range 5–50 μmol ml^{-1}. All three assay procedures yielded the same fetal–maternal differences and the same absolute glucose concentrations. To exclude the possibility of the presence of an unusual sugar, whole blood extracts were analysed by gas–liquid chromatography[6]. Glucose was the only major sugar present in maternal and fetal blood; fructose, galactose, mannose, and ribose did not occur in measurable concentrations. The possibility of a significant amount of fructose was also excluded by enzymatic tests using fructose kinase and hexose isomerase to convert fructose to glucose-6-phosphate and then assaying the latter with glucose-6-phosphate dehydrogenase. Establishing the absence of fructose was considered important because of its reported occurrence in cetaceans[7]. Insulin was measured by radioimmunoassay with porcine insulin serving both as the antigen in the preparation of antibodies and as the standard[8].

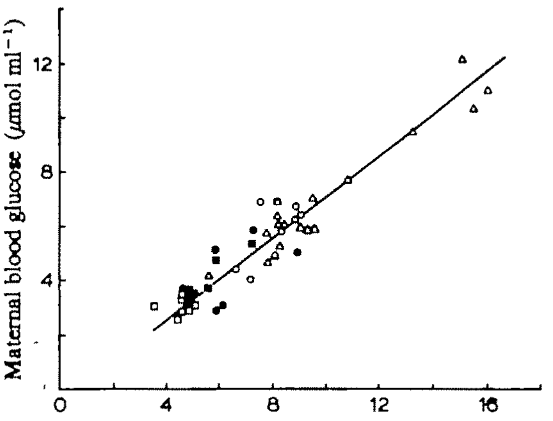

Fig. 1 A plot of glucose concentrations in whole blood of five maternal-fetal pairs. The different symbols refer to different pairs. Blood samples were taken before, during and after simulated diving. The highest glucose values were obtained after intravenous infusion of glucose.

Under resting and fasted conditions (in captivity for at least 12 h), maternal whole blood glucose concentrations ranged between 4 and 6 μmol ml^{-1} compared to 5–8 μmol ml^{-1} in fetal whole blood. Similar levels were found in samples drawn from undisturbed seals asleep on ice. Although the fetal concentrations were consistently higher than maternal ones, the two values tended to fluctuate in unison (Fig. 1).

To better understand how maternal and fetal blood glucose levels are regulated, a glucose load (1.5 g per kg of estimated maternal body weight) was infused over a period of 20 min into

Table 1 Fractional distribution of glucose between plasma and red blood cells in the maternal and fetal Weddell seal compared with the sheep

Species	Condition	Glucose (μmol ml^{-1}) Whole blood	Plasma	Red cells	Ratio plasma: red cells
Weddel seal (adult)	Pre-infusion	3.1	7	0.5	14
	60 min postinfusion	13 5	30	2.5	12
Weddell seal (fetus)	Pre-infusion	3.5	6	2.4	2.5
	60 min post-infusion	15.8	22.5	12.9	1.7
Sheep (adult)	Post-prandial		2.5	0.3	8.3
Sheep (fetus)			0.7	0.2	3.5

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the maternal circulation of three pregnant seals. In all three animals, the maternal concentration of glucose in both whole blood and plasma rose to values that exceeded those in the fetus throughout the infusion and for a short period after it was completed, but within 20–40 min fetal blood glucose levels were again higher than in the mother (Fig. 2*a*, *b*). The plasma concentration of insulin increased sharply in both mother and fetus ~15 min after the rise in glucose levels (Fig. 2*b*). In the fetus, the concentrations of plasma glucose and insulin followed similar patterns but in the mother, insulin levels remained elevated for at least 5 h after glucose infusion although glucose concentrations were falling.

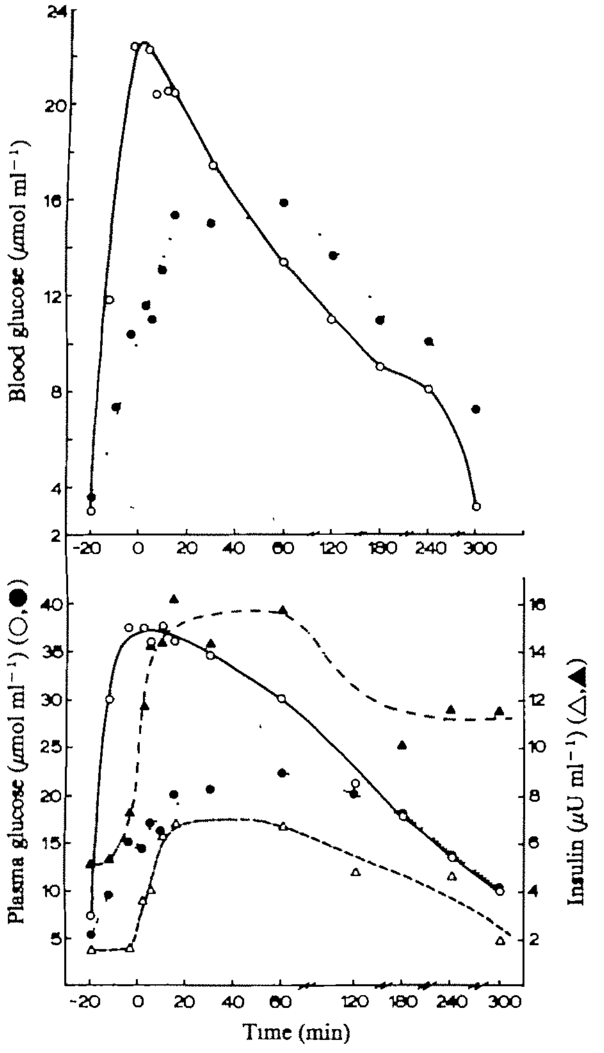

Fig. 2 *a*, Maternal (O) and fetal (●) concentrations of glucose in whole blood following intravenous infusion of glucose 1.5 g per kg. Time zero indicates the end of the 20 min period of infusion. *b*, Maternal glucose (O) and insulin (▲) and fetal glucose (●) and insulin (△) concentrations in plasma from the same glucose tolerance test as shown in (*a*).

As in other mammals, glucose levels are higher in maternal plasma than in fetal plasma and glucose moves from maternal to fetal blood down a concentration gradient. The unusually high glucose content of fetal whole blood relative to the mother arises from several factors. First, the gradient across the placenta is small (~2 μmol ml^{-1}) despite the use of arterial samples which would tend to exaggerate the apparent gradient. This is particularly so after glucose infusion when the gradient disappeared for 2 h (Fig. 2*b*). Second, the ratio of glucose concentrations in red cells to those in plasma in the mother is unusually low whereas that of the fetus is similar to other species (Table 1). Finally, the haematocrit of both mother and fetus is high (~60% and 70% respectively) which has the effect of increasing the ratio of plasma: whole blood concentration of glucose, especially in the mother.

Whereas a high haematocrit increases the O_2 carrying capacity of the blood, it clearly reduces the glucose carrying capacity although to a lesser degree in the fetus because of the greater entry of glucose into fetal red cells. The sustained hyperglycaemia after glucose loading (Fig. 2*b*) suggests that the rate of glucose turnover in the adult seal is low. Likewise, the small gradient of plasma glucose concentrations across the placenta suggests a low turnover rate in the fetus. Thus, neither mother nor fetus is likely to be disadvantaged by having a relatively low carrying capacity for glucose in the blood.

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Immunisation against gametes and asexual erythrocytic stages of a rodent malaria parasite

INVESTIGATIONS into the feasibility of vaccinating against malaria have centred mainly on the use of the sporozoite[1,2] or the asexual blood stages[3–6]. Immunisation using sexual stages of malaria parasites has been demonstrated with *Plasmodium gallinaceum* in chickens[7,8]. Gamete vaccines have been used to produce an effective immunity against transmission of the avian malaria parasite, with little or no effect on its asexual erythrocytic stages. More recently, this study has been extended to *P. knowlesi* infections in rhesus monkeys[9]; transmission-blocking immunity and resistance to asexual infection were obtained, but were fully effective only when the vaccine was combined with Freund's complete adjuvant. We report here successful immunisation of mice against the sexual stages of *P. yoelii* using formalin-fixed gametes. In addition to very effective transmission-blocking immunity, the vaccinated mice were strongly protected against asexual erythrocytic stages of the parasite.

Eight-week-old female outbred Theiler's Original (TO) mice were used as vertebrate hosts. The parasite, *P. yoelii nigeriensis*, was cryopreserved (stabilate reference LUMP 1316) soon after cyclical transmission through the vector *Anopheles stephensi*. Parasites from the first passage from the vector were used for initiating an infection.

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P. yoelii preferentially invades reticulocytes. Mice were made anaemic with three subcutaneous injections of phenylhydrazine and infected intravenously (i.v.) with parasitised red cells. As a result of the induced reticulocytosis, the parasitaemia rose to 70–80% with 0.5–1.5% of gametocytes. Mice were bled by cardiac puncture into 50 volumes of a suspended animation (SA) solution (0.21% Tris, 0.96% NaCl and 0.2% glucose, adjusted to pH 7.4) in which gametogenesis was reversibly suppressed[10]. The suspension was centrifuged at 500g for 10 min and the red cells were resuspended in a gamete-releasing medium[8] (2.5 vol of 5% NaCl, 2.5 vol of 10% glucose, 20 vol of 1.46% $NaHCO_3$ and 100 vol of inactivated fetal calf serum, adjusted to pH 8.0) in a proportion of 1:3 v/v, and the pH readjusted to 8.0. After 30 min at room temperature gametogenesis was complete and the suspension was centrifuged at 500g for 10 min. This procedure was repeated four times, resuspending the packed material in SA solution, so as to recover as many free gametes as possible. The pooled supernatants were centrifuged at 18,000g for 20 min and the pellet containing gametes was suspended in a solution of 1.0% formalin for 30 min at room temperature. The gametes were washed three times in SA solution at 18,000g. After the final wash, counts were made on a haemocytometer using a phase contrast microscope, and adjusted such that 0.2 ml contained 2×10^7 male gametes.

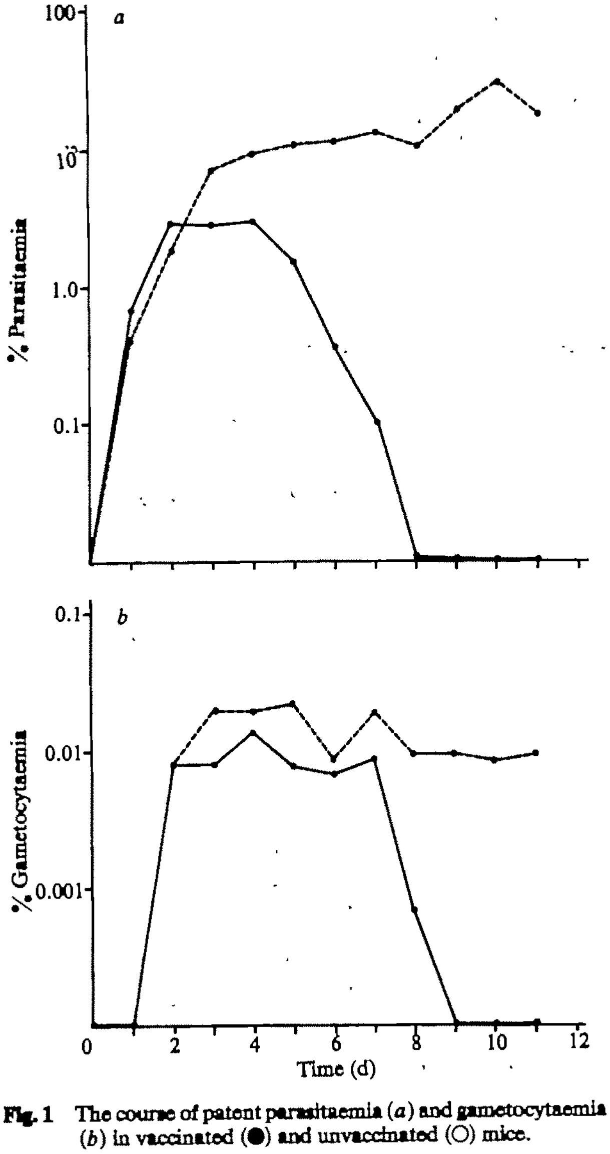

Fig. 1 The course of patent parasitaemia (*a*) and gametocytaemia (*b*) in vaccinated (●) and unvaccinated (○) mice.

Table 1 Oocyst production in mosquitoes fed on vaccinated and unvaccinated mice

Day	Unvaccinated mice No. of oocysts per 10 mosquitoes Expt 1	 2	 3	 Mean no. of oocysts per mosquito	Vaccinated mice No. of oocysts per 10 mosquitoes Expts 1, 2 and 3
1	0	0	0	0	0
2	114	1,638	2,162	130.4	0
3	496	498	1,287	76.0	0
4	1,406	40	0	48.2	0
5	0	62	0	2.0	0
Total counts	2,016	2,238	3,449		

In each experiment, 3 vaccinated and 3 unvaccinated mice were given a blood-induced challenge infection. Batches of mosquitoes were fed on each group of mice on days 1–6 of the infection. The mosquitoes were maintained at 24 °C and oocyst counts were done 1 week later.

The vaccine contained, in addition to male and female gametes, asexual parasites which under the light microscope appeared to be free of their host red cells, and occasional red cell 'ghosts' and white cells. 0.2 ml of the vaccine contained 2×10^7 male gametes, $6–8 \times 10^6$ female gametes and $10^5–10^6$ asexual parasites. 2×10^7 male gametes were obtained from 5.0 ml of infected blood. Each mouse received three doses of the freshly prepared vaccine i.v. at weekly intervals, that is, a total dose of 6×10^7 male gametes. One week after the final dose of the vaccine, vaccinated mice and an equal number of age-matched control mice were inoculated i.v. with 2×10^5 parasitised red cells. Thin blood films were made daily, stained with Giemsa and the parasitaemia and gametocytaemia ascertained. On days 1–6 after challenge, mosquitoes were fed on vaccinated and control mice, one cage per group. Any unfed female mosquitoes were removed from the cages and 1 week after feeding, 10 mosquitoes from each cage were dissected and the midguts examined microscopically for oocysts.

Results were obtained from three separate experiments involving nine vaccinated and an equal number of control animals. The infection was fatal in all but one of the control mice. Death of the control mice occurred either on days 6 and 7, with parasitaemias of 20–30% (Fig. 1*a*) or on days 18 and 19, with peak parasitaemias of 50–60%. In contrast, the vaccinated mice were apparently aparasitaemic by day 12, with an average clearance time of 8.8 d. The highest parasitaemia recorded in the vaccinated mice was 3.1%, reached on day 4 (Fig. 1*a*). The average gametocyte counts are represented in Fig. 1*b*. Mosquitoes fed on control mice had high total oocyst counts, these being derived mainly from feeds taken 2, 3 or 4 d after challenge. Mosquitoes fed on vaccinated mice showed no oocysts at all in their midguts throughout the infection (Table 1).

These experiments demonstrate a very effective immunity which blocks transmission. It does not require the use of an adjuvant and we are now investigating whether fewer gametes and/or smaller numbers of immunising doses can be used; preliminary studies indicate that a single dose can be equally effective. Immunity to asexual erythrocytic stages, though not complete, was also pronounced, manifested by a significant lowering of parasitaemia and a 100% survival as compared to the control mice, in which the parasitaemia was higher and the infection fatal in almost all of the mice. Gwadz and Green[9] used a gamete/trophozoite antigen mixture in their experiments with *P. knowlesi* and this also induced immunity against asexual parasites. There are two possible explanations. First, contamination of the gamete vaccines with asexual stages might be responsible. Experiments by Playfair *et al.*[6] and our own experience (unpublished) with saponin-lysed, formalin-fixed asexual stage parasites used as a vaccine without an adjuvant, have resulted in an effective immunity to blood-induced *P. yoelii* infections. The alternative proposition would be that gametes protect against asexual stages because they have protective antigens in common.

It is encouraging that such an effective block on transmission is possible by vaccinating with a relative crude antigen preparation, and without the need for an adjuvant. Further improvements both in methods of vaccine preparation and in its presentation are possible.

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Changes in ocular dominance induced in monocularly deprived lambs by stimulation with rotating gratings

AMBLYOPIA in children aged about 6 yr can be rapidly diminished by stimulating the amblyopic eye alone with rotating disks of square-wave gratings[1]. Such exposure for four periods each of 7 min has brought many of the patients up to 6/6 acuity, in some cases, after 'patching' of the good eye had been largely ineffective. However, in children of this age, the 'critical period' has probably passed. We decided to study the effects of presenting rotating gratings during the critical period, when the visual system is known to be acutely sensitive to visual deprivation. Could changes in the ocular dominance of cortical neurones be induced even in the relatively unfavourable conditions of moderate to light anaesthesia? We report here that monocular deprivation of 2–3 d in young lambs results in a marked decrease in the number of cells in the primary visual cortex which can be stimulated through the deprived eye, but that this can be rapidly increased by subsequent brief exposure of the deprived eye to a rotating square-wave grating. We chose to work on lambs and a pygmy goat in which one eye had been closed for a few days shortly after birth because we thought that such treatment would leave few cells driven initially by the deprived eye. As the visual cortex at that time is showing its greatest plasticity, it seemed likely that short exposures to the gratings would then have their greatest effect on the ocular dominance. Others had used repeated stimulation by moving bars on single cells of normal animals, but had obtained rather slight and transient effects[2]. We hoped to obtain reasonable changes in eye dominance during the limits of single acute experiments. Seven animals were used. They had normal binocular visual experience until the 4th day of life and were then monocularly deprived for either 2 (nos 1–4) or 3 (no. 5) d, after which they were anaesthetised and used for the experiment.

In the earlier experiments we anaesthetised the lambs and pygmy goats with 2% halothane followed by intravenous (i.v.) pentobarbitone, 10 mg per kg per h, for surgical anaesthesia, and 0.5–3.0 mg per kg per h pentobarbitone during recording. Although we gave the least pentobarbitone necessary during preparation for full anaesthesia, the blood pressure usually began to fall in these animals within 3–4 h and i.v. dextran was only temporarily effective in raising the blood pressure. The animal tranquilliser acetylpromazine (Crookes) was used to reduce the total dose of barbiturate given, but infusion was often necessary to maintain blood pressure. This had no observable effect on cortical responses and it does not cross the blood–brain barrier. In later experiments we used Althesin (Glaxo) 0.1 ml per kg, i.v., repeated as required, for surgical anaesthesia, followed for recording by 0.5–3.0 mg per kg per h pentobarbitone with 60% O_2, 40% N_2 mixture. The blood pressure in these experiments was well maintained without other assistance, and the cortical responses during the earlier hours of recording were much brisker. These difficulties are peculiar to lambs and kids between 3 and 14 d after birth.

To prevent large sampling errors, we avoided penetrations normal to the surface. Penetrations on the medial side of the ectolateral sulcus gave tracks in V1 of up to 3.5 mm long, usually parallel to the cortical surface. On average we sampled from six

Table 1 Summary of ocular dominance distribution in the seven experimental animals

Animal no.	Days after birth Left eye shut	Mapped		No. of units in ocular dominance groups 1–7 1	2	3	4	5	6	7
1 (L 27)*	4	15	Pre-stimulation	0	0	0	0	1	8	33
			Post-stimulation	5	5	1	2	4	10	28
2 (L 28)	4	22	Pre-stimulation	0	0	0	0	1	5	35
			Post-stimulation	1	4	0	7	3	23	69
3 (PG 2)	4	6	Pre-stimulation	6	0	2	5	9	9	33
			Post-stimulation	6	6	5	7	11	14	16
4 (L 32)*	5	7	Pre-stimulation	3	0	2	5	3	6	27
			Post-stimulation	9	8	9	12	11	19	33
5 (L 33)*	6	8	Pre-stimulation	2	1	1	7	12	14	33
			Post-stimulation	29	6	9	4	2	20	29
6 (L 34)*	7	9	Pre-stimulation	7	2	4	19	4	21	20
			Post-stimulation	15	10	8	8	3	14	8
7 (L 30)	6	8	Pre-control	1	1	0	1	3	9	15
			Post-control	0	2	1	2	1	4	8

A χ^2 test was used to test significance. L, Lamb; PG, pygmy goat.
* The difference between the pre- and post-stimulation is significant at $P \leq 0.01$.

ocular dominance columns for each sample of 60–100 cells both before and after stimulation by the gratings. All ocular dominance distributions seen in animals deprived for 2–3 d during the first week of life give very similar patterns (Table 1). Also, collecting 100 cells takes about 5 h, and there is no statistical difference between the distribution of dominance in the first 50 and the last 50 cells collected, so that neither sampling nor the passage of time disturbs the distribution.

Single unit recordings were obtained from the striate cortex contralateral to the deprived eye, for in a normal animal in this hemisphere the experienced eye would chiefly excite only 20% of the cells (ocular dominance groups 5–7, Fig. 2*a*. Tungsten-in-glass microelectrodes with impedances 1–5 M at 1,000 Hz were used[3]. Visual stimuli were hand-held targets moved against a screen 90 cm from the eyes. Unit properties such as orientation specificity (through each eye) and receptive field type were recorded, but we concentrated mainly on ocular dominance, that is, the degree to which a cell could be driven through one eye or the other.

Whether the responses were from visual areas 1 or 2 (V1 or V2) was determined during the experiment from the visuocortical map produced and subsequently verified in histological sections. In these animals the effects of monocular deprivation for 2–3 d are more marked in V1 than in V2, and so we describe only the units in V1. The presence or absence of spontaneously active but undrivable units was also noted.

Except for the control animal (no. 7), the experimental procedure was the same for each of the other four lambs. The deprived eye was opened and a large sample of single units was obtained from V1 during the first few hours. The experienced eye was then closed and the originally deprived eye was stimulated with rotating gratings for periods of 45 to 90 min. Six different spatial frequencies (ranging from $\frac{1}{4}$ cycle deg^{-1} to 8 cycles deg^{-1}, see Fig 1) were presented sequentially in random order during this period, each frequency for about 5 min at a rotation speed of 2 r.p.m.. The whole procedure was then repeated at a faster rotation speed (4–6 r.p.m.). The grating was mounted on a circular disk and subtended at an angle of 35° at the eye. The deprived eye fixated the centre of the grating and was also its axis of rotation. The exact durations of stimulation varied between animals (see Fig. 1 legend).

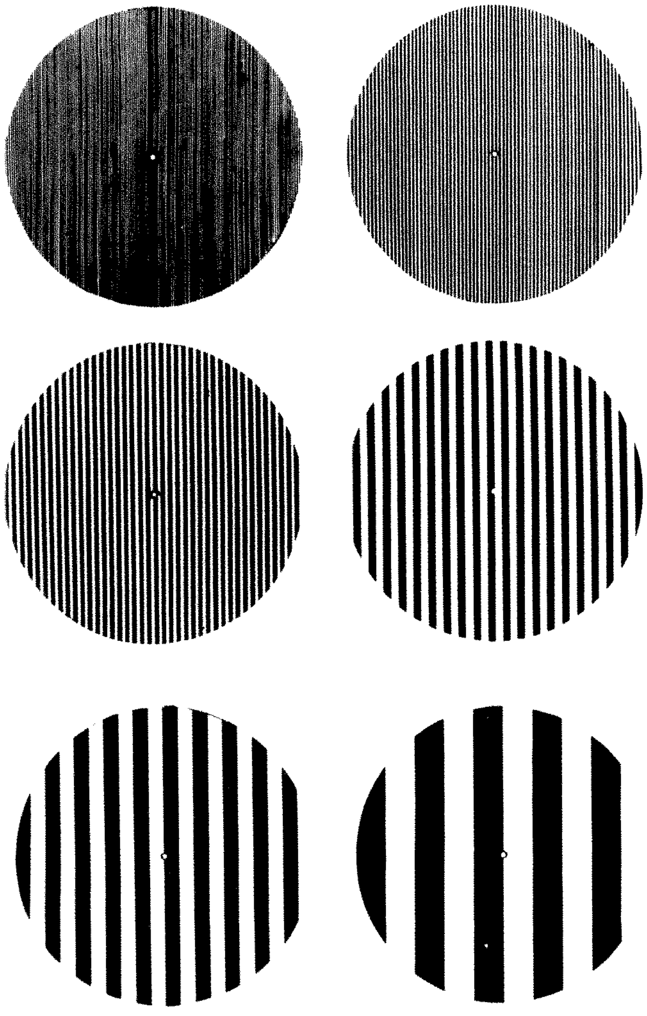

Fig. 1 High-contrast square-wave gratings used in the experiment to stimulate the deprived eye (see text). The spatial frequencies used were 8 cycles deg^{-1} to $\frac{1}{4}$ cycle deg^{-1}.

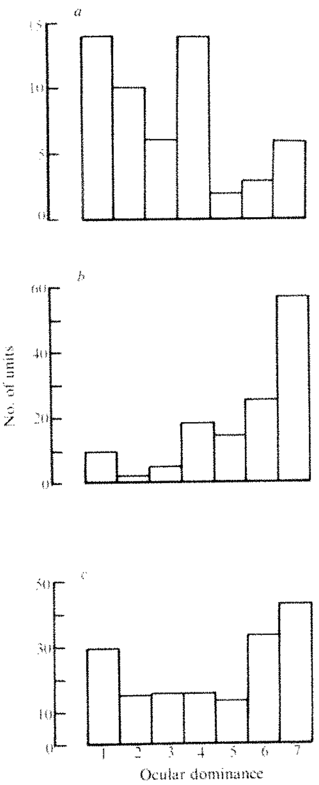

Fig. 2 Ocular dominance histogram of a normal lamb (*a*) and summed histograms of the four monocularly deprived animals used in the grating experiment and mapped within 10 d of birth; before (*b*) and after (*c*) stimulation. The convention of Hubel and Wiesel[7] is followed: group 1, driven only through the contralateral eye; 2, contralateral eye is much more effective than ipsilateral eye; 3, contralateral eye slightly more effective than ipsilateral eye; 4, the cell can be driven equally through either eye; 5, the ipsilateral eye slightly more effective than the contralateral eye; 6, the ipsilateral eye is much more effective; 7, can be driven only through the ipsilateral eye.

After grating presentation to the deprived eye, both eyes were closed for about 45 min to allow 'consolidation', as it has been reported that a short period of binocular deprivation may enhance the effects of selective visual experience[4]. Both eyes were then opened again and a new series of penetrations was made to obtain large samples of units. These data were then used for constructing the post-stimulation ocular dominance histograms. A striking shift in ocular dominance was produced by stimulation with rotating gratings. In the stimulated animals there was an increase in responsiveness of cortical cells to stimulation through the deprived eye, most marked in animal 5. These changes remained stable during the post-stimulation recording period (4–6 h).

Two control experiments were also carried out to determine whether the changes observed were specifically due to the presentation of gratings to the deprived eye. In lamb 7, the surgical procedure used was the same as in the other animals, but instead of presenting a rotating grating, the deprived eye was left open for 1 h (with the experienced eye closed) and was exposed to people walking around in the room. The pre- and post-control ocular dominance histograms in this animal were almost identical. Also, from ocular dominance determinations at the beginning and end of 10 h of repeated visual testing of the two eyes, it is clear that mere passage of time under anaesthesia, even with repeated visual testing, does not produce a reappearance of units dominated by the deprived eye.

Lamb 6 was also used for a control experiment. Our sample of neurones from the other four animals indicated the shift of ocular dominance to be expected from 2 d of monocular deprivation; usually about 6–9% of the total number of neurones were chiefly excited through the deprived eye (ocular dominance groups 1, 2 and 3) as compared with 50% in the normal lamb (Fig. 2*a*). In lamb 6, we opened the deprived eye and closed the experienced eye, and then allowed the animal to walk around the laboratory for 1 h 15 min to allow random visual stimulation of the initially deprived eye before anaesthetising the animal for recording. Several penetrations were then made to obtain a sufficiently large sample of neurones for the ocular dominance distribution.

The was a marked increase in the units driven equally well through either eye (ocular dominance group 4); this requires further investigation. However, the increase in cells dominated by the originally deprived eye was far less than that produced by exposure to the rotating grating for the same period in the other three animals. Hence, random visual stimulation of the deprived eye, even in a conscious animal, may be less effective than a rotating grating. This same animal was then exposed to a rotating grating with only the deprived eye open, and there was a sharp rise in the number of cells driven by the deprived eye.

It is not yet clear whether rotating gratings are the optimal stimuli for producing changes. Experiments on kittens suggest that changes in ocular dominance after reverse suturing begin only after 3 d of visual stimulation[5], and it would be of interest to replicate our experiments using kittens. However, we were not concerned to find the optimal stimulus to reverse the effects of deprivation, but rather, a rapid way of increasing the proportion of cells driven by the deprived eye. Banks *et al.*[1] found that the effect could be produced in children in whom the critical period had probably ended. In two later experiments in monocularly deprived lambs however, the effect could not be obtained after the end of the critical period. Although the revolving grating increases ocular dominance in our animals and improves visual acuity in patients, this does not imply that the stimulation is acting on the same parts of the visual pathway in both cases.

We therefore conclude that ocular dominance can be changed in periods at least as brief as 1 h, even in an anaesthetised preparation. The mechanism underlying this change in ocular dominance is at present obscure. Experiments are in progress to find out whether the effects of stimulating one portion of the visual field of the deprived eye with a grating may spread to adjacent regions within the same field, and not remain confined to the stimulated area alone. This would tell us whether some central diffuse excitatory mechanism is involved. The preparation would also be useful for studying such pharmacological effects as reported by Kasamatsu and Pettigrew[6].

Because of its rapidity of onset and long duration, this effect may provide a neurophysiological approach to understanding the nature of some long-term changes in the central nervous system.

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Dexamethasone-induced accumulation of a fibronectin and collagen extracellular matrix in transformed human cells

NUMEROUS differences have been demonstrated between normal and transformed cells in culture. One characteristic finding is the presence of a $2\text{–}2.5 \times 10^5$ molecular weight externally disposed glycoprotein (fibronectin) on normal cells and its absence from cell surface association in transformed cells (see refs 1–3 for reviews). Transformed human cells are known to synthesise and shed fibronectin, although at a much lower rate than comparable normal cells[4,5]. Studies using immunofluorescence or immunoperoxidase methods have demonstrated fibronectin in pericellular structures of fibroblasts and myoblasts in what we have termed an extracellular filamentous matrix[6]. In transformed cells that are producing fibronectin, though at a lower rate, there is a failure to incorporate the glycoprotein into the extracellular matrix. There is also a similar failure to incorporate collagen into a cellular matrix in transformed fibroblasts[7]. The function of this protein, fibronectin, is thought to be related to cellular adhesiveness and perhaps thereby have some role in cell shape. Transformed cells are less adhesive to each other and to substrates when grown *in vitro* and upon addition of exogenous fibronectin there is a concomitant increase in adhesiveness and change in cell shape[8,9]. There are many examples of glucocorticoids modulating differentiation some of which include, liver[10], pancreas[11], mammary gland[12], retina[13] and myoblasts[14]. Recent work in the exocrine pancreas[11] suggests that glucocorticoids may induce specific enzymes without altering the level of general cellular proteins. Glucocorticoids have been shown to stimulate proliferation in growth arrested cells[15] and modify in a synergistic or inhibitory manner cellular responses to other hormones. We report here the effects of dexamethasone on modifying one phenotypic characteristic of cells, namely the occurrence of an extracellular filamentous matrix consisting of fibronectin and collagen. Based on the old hypothesis that transformed or malignant cells are arrested in differentiation, we consider development of the matrix a manifestation of the differentiated state of fibroblasts.

Using the peroxidase–antiperoxidase (unlabelled antibody enzyme) method of immunocytochemical localisation, transformed cells are shown to lack an extracellular filamentous

Table 1 DNA levels of SV40 transformed human fibroblasts

Days of culture	Control (μg DNA per plate)	Dexamethasone (μg DNA per plate) 10^{-7} M	10^{-6} M
0	11.06	—	—
1	24.89	25.58	23.51
2	51.86	52.55	48.40
3	99.57	98.88	102.33

Cells were grown as described in Fig. 1 and DNA assay was performed as described previously[14].

matrix which reacts with fibronectin antibodies at either the light (Fig. 1*a*) or the electron microscopic level (Fig. 1*b*). Following treatment with 10^{-8}–10^{-4} M dexamethasone a distinct clustering of fibronectin forms around and adherent to these cells (Fig. 1*c*, *d*). This matrix begins to form as early as 24 h after treatment and becomes more pronounced with time in the presence of dexamethasone and increased cell density. The localisation appears most extensive at concentrations of 10^{-7}–10^{-6} M dexamethasone, whereas at 10^{-9} M, staining intensity drops to control levels where very little staining is seen as on untreated cells. Light and electron micrographs were taken following 3 d of treatment with dexamethasone. Ultrastructurally the pericellular localisation of fibronectin is seen in dexamethasone-treated cultures (Fig. 1*d*) where none existed in untreated control SV40 transformed human fibroblasts (Fig. 1*b*). When the specific antibody is adsorbed with fibronectin before reacting with dexamethasone treated cells, no reaction is seen, indicating the specificity of the reaction (Fig. 1*e*, *f*).

Using the peroxidase–antiperoxidase method we also observed the failure of transformed cells to produce a collagen matrix as seen at the electron microscopic level (Fig. 2*a*). Normal cells produce a well defined matrix of collagen that can be detected ultrastructurally with either the unlabelled antibody–enzyme method or ferritin coupled antibody (Furcht *et al.*, in preparation). When the dexamethasone-treated transformed cells (10^{-8}–10^{-4} M) are examined with immunocytochemistry using antibodies to procollagen type I (which are known to cross-react with collagen type I) collagen is also observed to form a fibrillar matrix as seen at the electron microscopic level (Fig. 2*b*). The concordance of the development of a matrix of fibronectin and collagen is quite similar to ultrastructural observations we have made using double antibody labelling at the ultrastructural level on normal human fibroblasts (ref. 16, Furcht *et. al.* unpublished).

We were next concerned about the effect of dexamethasone on cell proliferation and examined the effects of dexamethasone on DNA accumulation per day in cultures and found no difference between dexamethasone-treated (10^{-7}–10^{-6} M) and control transformed cells (Table 1).

A recent study has examined the effects of various hormones on fibronectin of the murine continuous cell line 3T3. The studies of Chen *et. al.*[17] show that when murine 3T3 cells are grown in low serum they lose fibronectin-like protein from their surface. It was further suggested that epidermal growth factor (EGF) could specifically induce the formation of a fibronectin-containing matrix on serum depleted 3T3 cells and that this effect was blocked by addition of antibodies to EGF. A variety of other hormones, including cortisol, were also tested which had no effect on fibronectin on 3T3 cells grown in low serum[17]. Our data differs in that we very clearly show an accumulation of fibronectin and collagen in transformed human cells treated with dexamethasone.

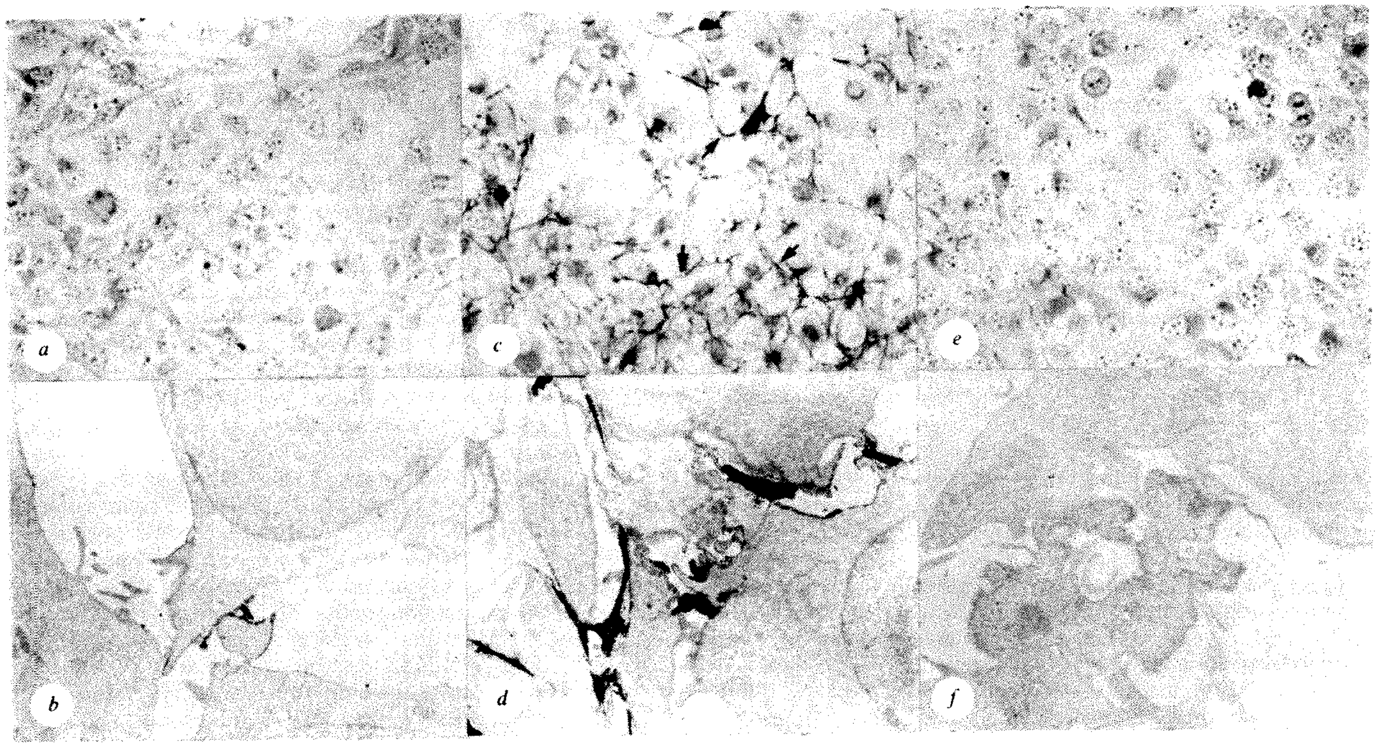

Fig. 1 Light (*a*,*c*,*e*) and electron (*b*,*d*,*f*) micrographs illustrating fibronectin accumulation on dexamethasone-treated SV40 transformed human fibroblasts. Cells are skin fibroblasts from a patient with Fanconi's syndrome which were transformed with SV40. The untransformed counterpart of the Fanconi fibroblasts has a normal distribution of fibronectin and procollagen type I identical to that seen in normal untransformed fibroblasts[16]. Cells were plated in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum. After one day medium was changed to either regular DMEM with serum supplemented with 10^{-9}–10^{-4} M dexamethasone. Three days after the medium change samples were fixed with 1% glutaraldehyde (Electron Microscopy Sciences) in phosphate buffered saline for 20 min at 37 °C. Fibronectin and collagen were localised using our modification of the unlabelled antibody–enzyme method described previously[6]. Fibronectin adsorbed antifibronectin was used as the primary antibody for adsorption controls. Adsorption of fibronectin antibody is as described[6]. Results presented are of cells treated with 10^{-7} M dexamethasone. Light micrographs are taken of whole cells grown on Falcon tissue culture dishes after reaction with antibodies and immunocytochemical staining using an Olympus BH microscope equipped with an OM-10 automatic camera. Cells are then embedded *in stiu* with Epon, thin sections cut, and viewed without counterstaining in a Philips EM 300 as described[6]. *a*, No fibronectin localisation is apparent on transformed human fibroblasts grown in DMEM with serum (×130). *b*, Ultrastructural fibronectin localisation of transformed cells grown in DMEM with serum. Sparse staining is seen at sites of cell–cell contact and in general is lacking from the cell surface (×3,800). *c*, A matrix of fibronectin localisation surrounds cells which have been treated with 10^{-7} M dexamethasone (arrows, ×130). *d*, A dense staining is seen between cells which were treated with 10^{-7} M dexamethasone (×3,600). *e*, No staining is seen if dexamethasone treated cells are reacted with fibronectin adsorbed antifibronectin as the primary antibody (×130). *f*, Fibronectin adsorption control. No staining is seen at the ultrastructural level (×3,400).

It is important to consider a number of points of difference between work by other laboratories and our studies where we use: (1) human cells; (2) transformed cells which do not make fibronectin in a matrix normally; (3) normal culture conditions of medium and serum. This is to be contrasted to the rather artificially induced loss of fibronectin which occurs in serum deprived 3T3 cells[17]. As fibronectin is routinely present on 3T3 cells, reproduction of its loss with serum restriction could be subject to numerous difficulties. The cell type of a 3T3 is really unknown with speculation ranging from an endothelial cell[18] to a fibroblast, with also the potential to differentiate into an adipose cell with lipid accumulation (3T3-LI)[19]. Furthermore, 3T3 cells will form tumours if they are attached to a substrate and injected into immunosuppressed animals, raising the question of the propriety of using these cells as 'normal' cells[20].

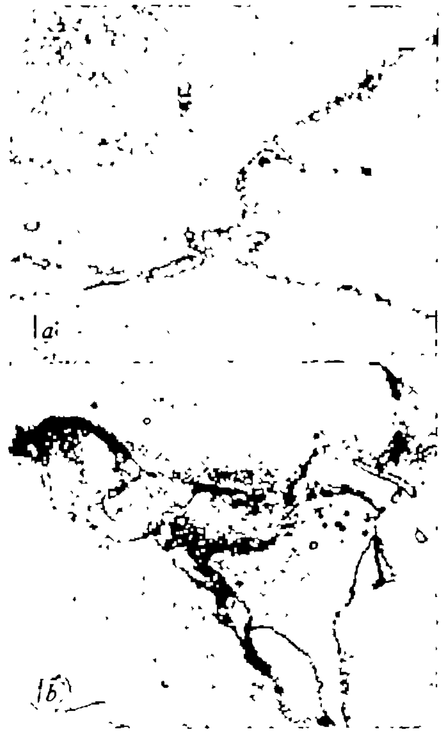

Fig. 2 Electron micrographs of thin sections illustrating immunocytochemical localisation of procollagen type I. Either affinity purified antibodies to procollagen I or the IgG fraction of antiserum to procollagen I was used with similar results. This antiserum also cross-reacts with collagen (Furcht *et. al.* submitted). Cells were treated as described in Fig. 1. *a*, Transformed human fibroblasts grown in DMEM lack a collagen matrix (×5400); *b*, transformed human fibroblasts grown in DMEM supplemented with 10^{-7} M dexamethasone form a collagen matrix identical to the fibronectin matrix seen in Fig. 1*d* (×5,400).

Several mechanisms should be considered for the dexamethasone stimulated accumulation of fibronectin and collagen associated on the cell surface of transformed human cells. First, recent studies have shown that the translatable mRNA for fibronectin and collagen is decreased in transformed cells[21] and perhaps dexamethasone is stimulating synthesis of this glycoprotein. Second, dexamethasone may effect whatever the processes are governing the polymerisation of fibronectin and adherence to cells so that more of the fibronectin and collagen synthesised remains or becomes adherent to cells. Based on the known interaction of fibronectin and collagen this might also involve collagen. Third, dexamethasone may modify the breakdown of fibronectin through modulating effects of hormones or proteases. With regard to this, surface associated fibronectin is very sensitive to protease degradation by trypsin and plasmin[22]. In relationship to this study, many transformed cells have elevated plasminogen activator levels[23] and other work has shown that dexamethasone will inhibit plasminogen activator levels in macrophages[24] and transformed cells[25]. Lastly, dexamethasone is known to modify prostaglandin biosynthesis[26], possibly by release of their precursor, aracadonic acid, or by some mechanism including transcription and translation. Therefore, prostaglandins in some unspecified way may be related to the dexamethasone-induced accumulation of fibronectin on transformed human cells.

In summary, if one postulates that the presence of fibronectin and collagen in normal substrate-attached fibroblast cells represents the differentiated state then the absence with transformation represents an arrest or blockade of differentiation. Based on this postulate it would then appear that dexamethasone treatment of transformed cells is capable of overcoming this arrest of differentiation as manifested in these two phenotypic traits, and results in the accumulation of fibronectin and collagen in transformed human cells.

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Possible behavioural function for noradrenaline–acetylcholine interaction in brain

IN the peripheral nervous system, acetylcholine (ACh) acts on presynaptic muscarinic receptors to inhibit the release of noradrenaline (NA) from nerve terminals and by way of nicotinic presynaptic receptors to stimulate release[1]. A NA–ACh interaction has also been postulated[2–7] to occur in the central nervous system (CNS). Cholinergic agonists decrease the levels of NA in various brain areas[5,6], affect the release of NA in *in vitro* preparations[3,4] and have similar effects *in vivo* as measured by increased metabolites of NA (ref. 2). A reciprocal interaction, that of NA modulating ACh release in the CNS does not seem to occur, according to evidence[8] available at present. We report here that depletion of forebrain NA by intracerebral injection of the selective neurotoxin 6-hydroxydopamine (6-OHDA) almost completely blocks the catalepsy induced by cholinergic agonists such as pilocarpine and arecoline[9–12] and enhances the locomotor stimulation caused by the anticholinergic drugs scopolamine and atropine[13–15]. Although these actions have most usually been ascribed to an interaction with dopamine (DA) systems[16,17], our data show that functionally significant interactions between ACh and NA systems also occur.

Forebrain NA was depleted by intracerebral injection of the selective catecholamine neurotoxin 6-hydroxydopamine (6-OHDA)[18] into the dorsal bundle in which NA axons course from their cell bodies in the pontine nucleus locus coeruleus, to innervate wide areas of the forebrain such as cortex and hippocampus[19,20]. The extent of the depletion achieved is shown in Table 1 for a representative group of animals used in the following behavioural studies. Post-mortem assay[21] of endogenous amines revealed that cortical and hippocampal NA were depleted to less than 5% of control values, and that hypothalamic NA was also severely reduced, but with little effect on brain dopamine or descending NA systems.

The cataleptic effect of cholinergic agonists pilocarpine and arecoline is shown in Fig. 1. Each point represents a different group of 10 control and 10 lesioned rats, as the effects of arecoline are reported to change with repeated testing[11]. Catalepsy was measured by placing the animal's forepaws on a horizontal bar 7.5 cm above the ground and timing the period before the animal removed one or both paws to the ground. Saline-injected rats typically remove their paws within 10–15 s (Fig. 1). Both arecoline and pilocarpine induced considerable catalepsy in control animals, with times of up to 50 s or 100 s being required to remove the paws. Such catalepsy was almost totally blocked in the NA-depleted animals. Significant differences[22] were found between control and lesioned groups at all doses used, except 5 mg per kg of arecoline, which failed to produce catalepsy even in controls. The catalepsy induced by haloperidol, a dopamine-receptor blocking agent failed to differ at any dose[23] used, in spite of the induction of considerable catalepsy in both control and lesioned groups. This suggests that noradrenergic interaction is specific for cholinergic mechanisms, since the lesion fails to alter either dopamine levels (Table 1) or dopaminergic function as tested by haloperidol (Fig. 1).

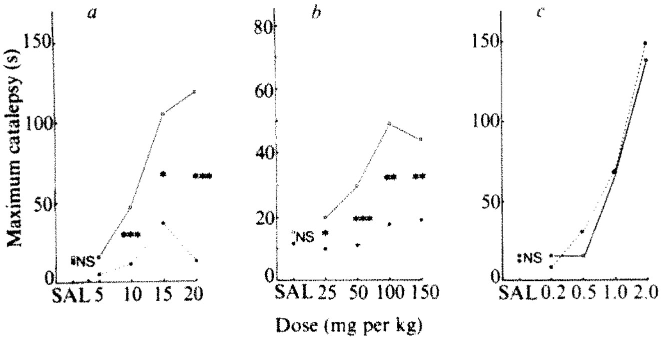

Fig. 1 Catalepsy induced in control and 6-hydroxydopamine lesioned rats. 4 μg 6-OHDA base (Regis, 6-OHDA HBr) dissolved in 2 μl of 0.9% saline with 0.2 mg ml^{-1} ascorbic acid, was infused through a 34 gauge cannula bilaterally at AP + 2.6 mm, ML ± 1.1 mm and DV + 3.7 mm from interaural zero, with the skull level between lambda and bregma. Controls received infusion of saline-ascorbic vehicle. Each point represents the mean of 10 control (□) or 10 lesioned (■) rats and fresh groups of animals were used for different points on the dose–response curve of a given drug. *a*, Arecoline; *b*, pilocarpine; *c*, haloperidol. The drugs were dissolved in 0.9% saline and injected intraperitoneally (i.p.). In the case of pilocarpine, 1 mg per kg methylscopolamine was given i.p. 30 min before the pilocarpine, to block the peripheral effects of the drug which interfere with catalepsy measurement. Control and lesioned groups differed at the following levels of significance using a two-tailed Mann-Whitney *U* test, *5%, **1%, ***0.1%, NS, not significant.

Table 1 Postmortem assay of amine concentrations in control and 6-hydroxydopamine lesioned rats

Region	Control ($n = 5$)	Lesioned ($n = 5$)	%*
Noradrenaline			
Hippocampus–cortex	264 ± 6	6 ± 1	2
Hypothalamus	2,240 ± 77	590 ± 87	26
Cerebellum	219 ± 12	271 ± 8	124
Spinal cord	255 ± 6	307 ± 12	120
Dopamine			
Striatum	13,170 ± 570	11,570 ± 1,190	88

Values are means in ng amine per g tissue, with s.e.m.

* Percentage of control concentrations remaining in lesioned tissues.

Cholinergic (muscarinic) antagonists such as scopolamine and atropine induced a mild locomotor stimulation. This was measured in photocell activity cages for a period of 2 h following drug injection. It can be seen from Fig. 2*a* and *b* that the representative doses used induced significant activity in controls compared with saline injections and that this waned with time. Although the initial locomotor stimulation over the first hour did not differ between controls and lesioned rats, an enhancement of the stimulant effect was evident in the second hour of testing. This was a central effect, since methylscopolamine, which does not cross the blood–brain barrier, failed to induce locomotor activity even in controls (Fig. 2*c*). Again, the noradrenergic interaction is specific to cholinergic systems, as the indirect dopamine agonist *d*-amphetamine, which is known to act through dopamine systems to cause locomotor activity[16,17], did not differ between control and lesioned groups (Fig. 2*d*).

The results suggest that cholinergic drugs modulate the activity of a noradrenergic system the function of which may be to influence the degree of behavioural arousal, as reflected by catalepsy at one extreme and locomotor stimulation at the other. Based on changes in sleep–wakefulness after 6–OHDA lesions, similar suggestions regarding the function of ascending noradrenergic neurones have been made by Lidbrink[24,25]. These behavioural findings also supplement biochemical observations that ACh can increase the release of NA in the CNS[2–7].

We tentatively propose the following model for the NA–ACh interaction. There is evidence for excitatory muscarinic receptors on noradrenergic cell bodies in the brain stem. It is known from microiontophoretic studies that the locus coeruleus has excitatory ACh receptors on its cell bodies[26], and may receive cholinergic input from neighbouring cell groups[27–29]. Cholinergic stimulation in the vicinity of the locus coeruleus has also been found to induce atonia and catalepsy[30–32]. In our model, the increased terminal release of NA that would result from the

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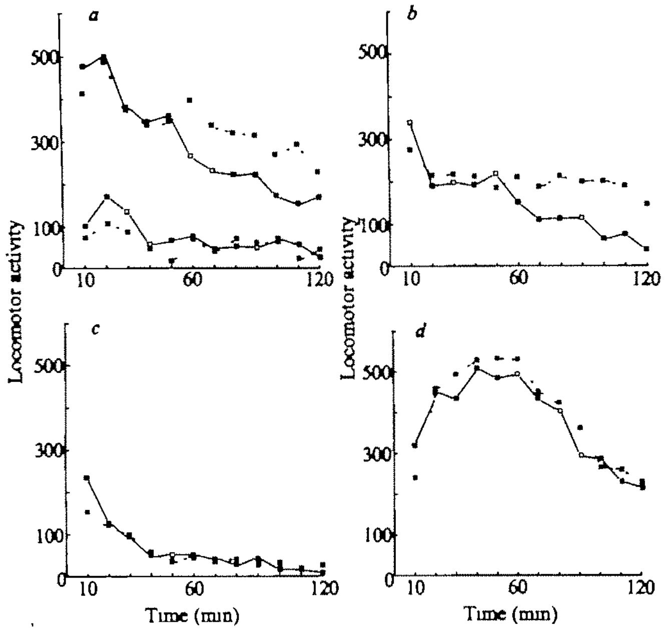

Fig. 2 Locomotor stimulant effect induced in control and 6-hydroxydopamine lesioned rats. Values are mean photocell beam interruptions per 10 min for 10 controls (□) and 10 lesioned (■) rats. *a*, 1 mg per kg scopolamine (upper lines), saline (lower lines). *b*, 10 mg per kg atropine. *c*, 1 mg per kg methylscopolamine; *d*, 1 mg per kg amphetamine. All drugs were dissolved in 0.9% saline and injected i.p. following 1 h of habituation to the photocell cages. Control and lesioned rats were compared statistically by a repeated measures analysis of variance.

cholinergic stimulation of the NA perikarya is a critical factor in producing the cataleptic response. Conversely, muscarinic antagonists would block the endogenous cholinergic activation of the NA cell bodies, thereby reducing the normal NA release, resulting in motor stimulation; a behavioural state opposite to catalepsy. The model might predict that dorsal bundle lesions would, by themselves, increase locomotor activity. That they do not, suggests that the anti-cholinergic drugs increase locomotor activity by actions on other neuronal systems in addition to the ascending NA projections. That is, rather than being due to decreased NA release alone, the locomotor stimulation induced by anticholinergic drugs seems to be a combined result of decreased NA release and actions on other, presently unspecified, systems. Similarly, although the ascending NA projections seem to play an essential part in muscarinic agonist-induced catalepsy, it is likely that concomitant cholinergic stimulation of other neuronal systems also contributes to this behaviour.

Our results demonstrate for the first time the existence of functionally important interactions between central cholinergic and noradrenergic mechanisms, suggest a role of the noradrenergic system itself and delineate a possible locus of this interaction.

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Reduction in benzodiazepine receptors associated with Purkinje cell degeneration in 'nervous' mutant mice

HIGH affinity benzodiazepine binding sites which are both stereospecific and saturable[1–3] have recently been characterised in the mammalian central nervous system (CNS). The good correlations obtained between the binding affinities of a series of benzodiazepines and their potencies as anticonvulsants, anxiolytics and muscle relaxants strongly suggest that these sites may function as pharmacological receptors for the benzodiazepines[1,4]. Although both the gross anatomical[3,5] and subcellular distribution[6] of these sites have been described, the cellular localisation of these sites is not known, nor has a direct functional link been established between benzodiazepine binding sites and the neuropharmacological effects of these drugs. We now report that 'nervous' mutant mice, which undergo a selective loss of more than 90% of their cerebellar Purkinje cells during maturation[7,8], have a marked reduction in the number of cerebellar benzodiazepine receptors. This loss in benzodiazepine receptors seems to be confined to the cerebellum, and is not accompanied by an alteration in the affinity of the receptor for diazepam. These data clearly demonstrate the loss of benzodiazepine receptors in a cell type which has been shown to be both neurochemically[9] and electrophysiologically[10] sensitive to benzodiazepines, thus providing evidence that benzodiazepine receptors function in the pharmacological actions of these drugs.

Nervous (*nr*/*nr*), littermate (*nr*/+), and normal (+/+) mice of the C3HeB/FeJ strain (Jackson Laboratories), 9–11 weeks old, were used in these studies. Nervous mice were easily distinguished from littermates by a smaller size and characteristic ataxic gait. In addition, cerebellar weights were significantly reduced in the nervous mice when compared to littermate controls or normal mice (36 ± 2.5 mg compared with 55 ± 5.7 and 56 ± 5.0, respectively). No differences in forebrain weight were noted among the three groups. Mice were killed by decapitation, and the forebrains and cerebella dissected, weighed, and homogenised in 9 volumes of ice-cold Tris buffer (50 mM, *p*H 7.4). Incubation mixtures consisted of 40 μl of the 10% (w/v) tissue homogenate, 360 μl of Tris buffer, 50 μl of ^{3}H-diazepam (79.8 Ci mmol^{-1}, NEN, diluted in Tris buffer), and 50 μl of water or unlabelled diazepam (final concentration 3 μM). Nonspecific binding, defined as the amount of ^{3}H-diazepam binding to tissue in the presence of 3 μM diazepam, was less than 10% total binding using concentrations of diazepam near the K_d (compare refs 1, 11). Incubation conditions and

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Table 1 Characteristics of ^{3}H-diazepam binding in 'nervous' (*nr*/*nr*), littermate (+/*nr*), and control (+/+) C3HeB/FeJ mice

	^{3}H-diazepam binding* (fmol per mg protein)		K_d (nM)		B_{max} (fmol per mg protein)	
	Cerebellum	Forebrain	Cerebellum	Forebrain	Cerebellum	Forebrain
Control (+/+)	415±10 (4)	424±16 (4)	4.4	4.6	957	1,110
Littermate (+/*nr*)	398±12 (4)	440±21 (4)	4.9	4.4	979	1,023
Nervous (*nr*/*nr*)	295±28 (4)†	390±24 (4)	3.9	4.5	668	1,075

Values are means ±s.e.m. with the number of animals in parentheses. Values for the K_d and B_{max} were obtained from the pooled tissue of seven mice per group.

* Binding obtained with 3.5 nM ^{3}H-diazepam.

† $P < 0.02$ compared to littermates, $P < 0.01$ compared to control mice.

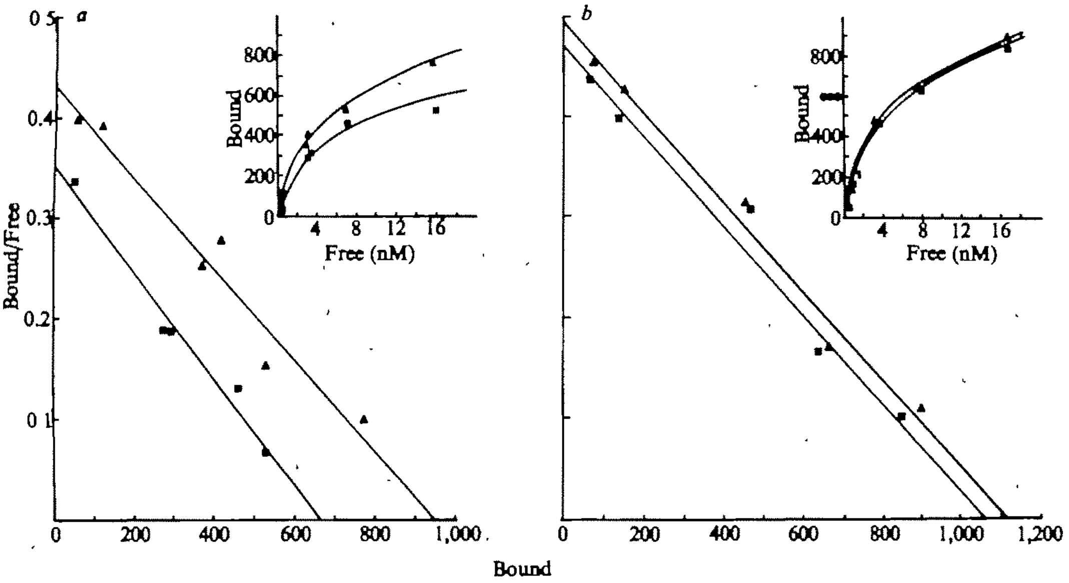

Fig. 1 Kinetics of ^{3}H-diazepam binding in nervous, littermate, and control mice: Scatchard analyses of binding in nervous (■) and control mice (▲) in *a*, cerebellar and *b*, forebrain homogenates. Insets: saturation plot. Scatchard and saturation analysis of littermate (*nr*/+) (■) and control (+/+) (▲) mice resulted in nearly superimposable curves (compare Table 1).

materials used for determination of ^{3}H-diazepam binding have been described elsewhere[2,11].

Preliminary experiments were performed using 3.5 nM ^{3}H-diazepam, as this ligand concentration would reveal differences in either receptor affinity or number. The binding of ^{3}H-diazepam in cerebella of nervous mice was reduced 25 and 29% when compared with littermate and normal C3HeB/FeJ mice ($P < 0.02$ and < 0.01, respectively) (Table 1). In contrast, no significant changes in ^{3}H-diazepam binding were observed in forebrain homogenates among the three groups (Table 1). Scatchard analysis (Table 1, Fig. 1*a*) of ^{3}H-diazepam binding revealed a marked reduction in the maximum number of benzodiazepine binding sites (B_{max}) in nervous mutant cerebella, with no apparent alteration in receptor affinity for ^{3}H-diazepam (K_d) (Table 1, Fig. 1*a*). Both the B_{max} and K_d for ^{3}H-diazepam binding were nearly identical among the three groups of mice in forebrain homogenates (Table 1, Fig. 1*b*). The reduction in benzodiazepine receptors observed in the nervous mice was manifest whether the results were expressed on a wet weight or mg protein basis.

Benzodiazepines have profound neurochemical and electrophysiological[10] effects on cerebellar Purkinje cells, presenting a model to assess the relationship between benzodiazepine receptors and the pharmacological effects of these drugs in a specific cell type.

The dramatic reduction in benzodiazepine receptors observed in the cerebella of nervous mice, which suffer a selective loss of Purkinje cells, clearly identifies these cells as possessing both a large number and high density of receptors, as Purkinje cells represent only a minute fraction of the total cerebellar neurone population. In contrast, 'weaver' mutant mice which undergo a selective loss of cerebellar granule cells, have been found to have no reduction in cerebellar benzodiazepine receptors (S. Snyder, personal communication). Heterozygous littermates (*nr*/+) have binding characteristics indistinguishable from control animals (+/+), demonstrating the necessity for the phenotypic expression of this defect to be manifest (degeneration of Purkinje cells) for a loss in benzodiazepine receptors to occur. The reduction in benzodiazepine receptors in cerebella of nervous mutant mice represents the first reported degenerative genetic disorder linked to altered numbers of receptors in the CNS. Furthermore, these observations strongly support a direct functional association between benzodiazepine receptors and the pharmacological actions of these drugs in the CNS.

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Opiate antagonist reverses the cardiovascular effects of inhalation anaesthesia

THAT opiate receptors may mediate the action of inhalation anaesthetics is evident from the fact that intravenous injection of naloxone decreases the pain threshold in rats during inhalation anaesthesia[1,2]. But inhalation anaesthetics have not only analgesic and hypnotic properties, they also cause undesirable depression of the cardiovascular system. To investigate whether opiate receptors mediate the cardiovascular response to inhalation anaesthesia we perfused continuously the cerebro-ventricular system of both conscious and anaesthetised dogs with naloxone. Effects of anaesthesia with and without naloxone perfusion were compared for blood pressure, heart rate and baroreflex activity. The constant perfusion technique was adopted to avoid the drastic transients of naloxone brain concentrations which occur following intravenous naloxone application[3,4] and which might have masked the naloxone effects in recent reports[5–7]. We show here that (−)naloxone in contrast to (+)naloxone is capable of antagonising the circulatory effects of halothane anaesthesia from the fourth cerebral ventricle.

Eight dogs were prepared for perfusion of their cerebroventricular system under general anaesthesia. For perfusion of the fourth ventricle five dogs were used in whom stainless steel cannulae were permanently implanted, one into the rostral end of the fourth cerebral ventricle (inflow cannula) and another one into the cisterna cerebello-medullaris (outflow cannula) which drains the cerebral spinal fluid from the fourth ventricle. For perfusion of the third ventricle three other dogs were prepared with two cannulae located in each lateral ventricle. Additionally, both common carotid arteries were exteriorised into skin loops for direct blood pressure recording and also for testing of the carotid sinus reflex response to bilateral clamping of the loop arteries.

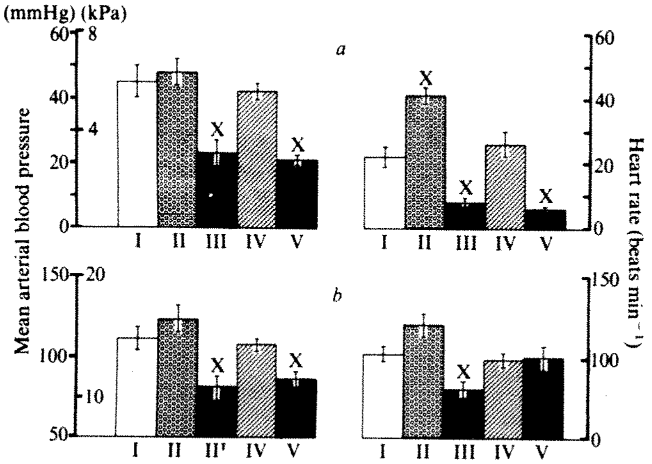

Fig. 1 Effects of continuous perfusion of the fourth cerebral ventricle without and with (−)naloxone at a concentration of 20 μg ml^{-1} on blood pressure, heart rate and carotid sinus reflex activity in conscious and anaesthetised (halothane) dogs. Perfusion periods are always 20 min. Halothane anaesthesia in combination with perfusion of the ventricle with Ringer's was maintained for 60 min. *a*, Response to clamping of carotid arteries: *b*, values before clamping. I, Conscious + Ringer, 20 min; II, conscious + naloxone, 20 min; III, anaesthetia 0.75 vol%, 60 min; IV, anaesthetia + naloxone, 20 min; V, anaesthesia 1.37 vol%, 20 min. Averages (± s.e.m.) from 10 experiments in three dogs. Differences relative to the controls (column I) were tested with Student paired *t*-test (X, $2P < 0.005$).

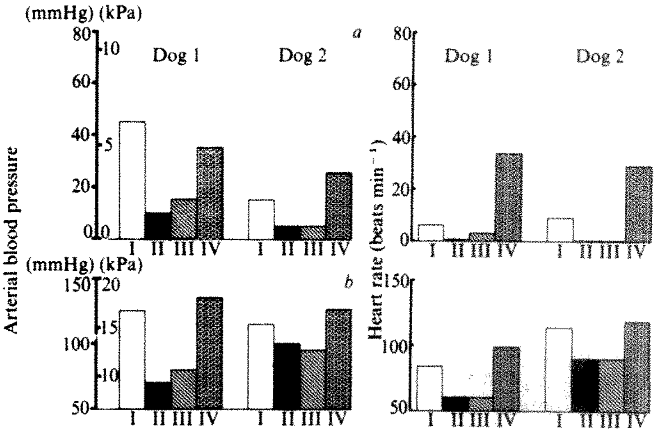

Fig. 2 Comparison of the effects of (+)naloxone and (−)naloxone at a concentration of 20 μg ml^{-1} perfused through the fourth cerebral ventricle on halothane-induced arterial hypotension, bradycardia and depressed carotid sinus reflex activity in two dogs. *a*, Response to carotid clamping; *b*, values before clamping; I, conscious; II–IV, halothane anaesthesia 0.72 vol%; II, 60 min; III, (+)naloxone, 20 min; IV, (−)naloxone, 20 min. There is hardly any effect from (+)naloxone but (−)naloxone promptly reverses the halothane effects to levels of the conscious state.

The dogs were trained to lie quietly and were unrestrained in the lateral position for the experiments in the conscious state. The actual experiments were performed 4 weeks after surgery. The animals participated for several months in the study and were familiar with the experimental environment. All the dogs were then repeatedly subjected to the experimental procedure described in the legend to Fig. 1. Ventricular perfusion (0.15 ml min^{-1}) was achieved with a microdrive single-channel push–pull pump (Braun Melsungen Unica B II). The restriction of the perfusion to the respective ventricles was verified by post-mortem dye distribution studies in each dog. The animals were anaesthetised with halothane at concentrations of 0.72–0.82 vol% in pure oxygen. The individual concentration was adjusted to render the animal analgesic as tested by pinching its nose with a haemostat.

Halothane was administered by a calibrated vapouriser adjusted to keep the end-expiratory concentration of the anaesthetic constant, which was verified by mass spectrometry (Perkins-Elmer MGA 1100). The responses of the animals to repeated bilateral carotid clamping of 1 minute duration were evaluated from continuous recordings of the heart rate and the arterial pressure.

In conscious dogs perfusion of the fourth cerebral ventricle with (−)naloxone results in a small increase in blood pressure and heart rate in association with unchanged reflex response of blood pressure but clearly augmented heart rate reflex (Fig. 1, columns I and II). During anaesthesia in combination with ventricular perfusion with Ringer's solution without naxolone (column III) all parameters are conspicuously reduced. But, most important, when (−)naloxone was added to the perfusate all parameters came back to the levels of the conscious controls. In fact, except for the heart rate levels, there are no significant differences present between the naloxone effects at the end of the 20 minute perfusion period (column IV) and the conscious state (column I). Finally, about twice the halothane concentration is necessary to antagonise the naloxone action (column V).

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These observations suggest that structures bordering the fourth cerebral ventricle mediate the naloxone effect on inhalation anaesthesia. This is likely as (−)naloxone was found to be completely without influence on the circulatory responses in three dogs in whom the third and lateral cerebral ventricles were perfused under essentially the same protocol. Therefore, possible candidates for sites of interaction with naloxone are those opiate receptors located on the floor of the fourth cerebral ventricle and which continue into the periductal grey matter[8,10]. These receptors are located rather superficially and opiates bind intensively here[10], penetrating to about 800 μm after local application[11]. Thus, there are favourable conditions in our experiments for an interaction between naloxone and these receptors. Finally, proof for the specificity of these effects came from observations that (+)naloxone does not reverse the cardiovascular effects of halothane anaesthesia. This was shown in two dogs (Fig. 2) where perfusion of the fourth ventricle with (+)naloxone for 20 min had no discernible effects in the conscious state and did not reverse the arterial hypotension, the bradycardia and the depressed baroreflex activity of halothane anaesthesia maintained for 60 min. In contrast, all circulatory parameters promptly returned to the conscious control levels after a 20 min perfusion period with (−)naloxone.

Thus, it seems safe to conclude that naloxone antagonises the cardiovascular manifestations of inhalation anaesthesia by stereospecific binding with opiate receptors in structures bordering the fourth cerebral ventricle. Possibly, these are the same receptors which are located below the floor of the fourth cerebral ventricle from where opiates and morphinomimetic peptides trigger analgesia[13–15] and profound sedation with catalepsia[16,17].

Whatever the pharmacological mechanism behind the naloxone effect on inhalation anaesthesia might be, our observations strongly suggest that the opiate receptor system seems to be of importance for its mediation. If this is so, then it might be necessary to reconsider the currently held view that inhalation anaesthetics exert their pharmacological effects by nonspecific action on cell membranes. At least the cardiovascular manifestations of halothane anaesthesia are more likely to be the consequence of its interaction with opiate receptors in the brain rather than of nonspecific side-effects on the heart and/or blood vessels.

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Local anaesthetics inhibit tension development and Nile blue fluorescence signals in frog muscle fibres

FLUORESCENT DYES have been increasingly used to measure fluorescence intensity changes associated with changes in transmembrane potential in various preparations[1,2]. The use of these compounds is of particular interest in muscle fibres, as they can be used to detect potential changes occurring across membranes not directly accessible to electrical measurements, and possibly associated with excitation–contraction coupling processes. For example, muscle fibres stained with Merocyanine 540 and electrically stimulated, give an early fluorescent signal which has been thought to arise from surface and T-system membranes[3,4]. More interestingly, fibres stained with Nile blue A and electrically stimulated have been reported to give fluorescence signals probably arising from the sarcoplasmic reticulum membrane[2,5–7]. Optical retardation changes arising from this membrane system have also been reported[8,9]. These results seem to indicate the existence of an electrical response across the sarcoplasmic reticulum membrane which might be associated with calcium release from these structures during contractile activation. To test this possibility, we investigated and report here the effects of the local anaesthetics, procaine and tetracaine, on tension development and their comparison with the effects on the fluorescence signals generated in muscle

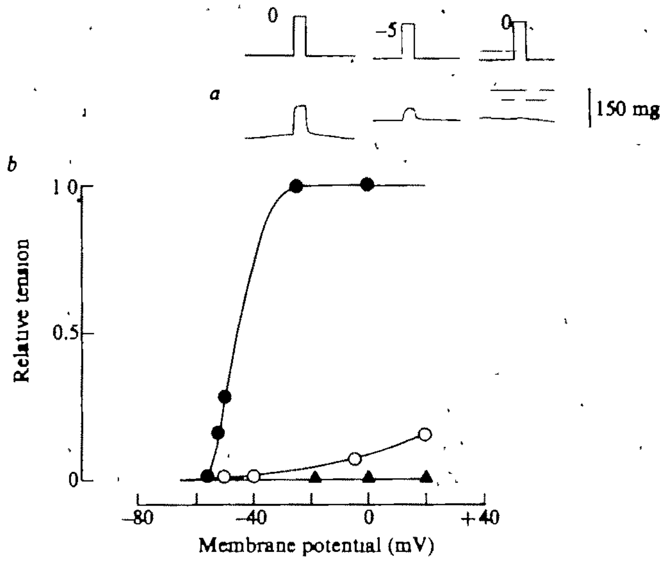

Fig. 1 Effect of tetracaine and procaine on the tension developed by three single fibres of approximately the same diameter (60–70 μm). The experiments used small bundles of short fibres (1–1.5 mm) dissected from the *Lumbricalis brevis* digit IV of the hindlimb of *Rana pipiens*. A two-microelectrode voltage-clamp technique was used to hold the membrane potential at a value of −100 mV and to apply depolarising pulses[13]. Tension developed by the clamped fibre was registered by means of an RCA 5734 transducer. The fibres were bathed in normal Ringer's solution containing 10^{-6} g ml^{-1} TTX to abolish sodium-dependent regenerative responses and improve the clamping conditions. *a*, From left to right, contractile responses (lower traces) to 1-s depolarising pulses (upper traces) obtained in normal Ringer, and in the presence of 5 mM procaine and 0.25 mM tetracaine, respectively. The level of the membrane potential during the pulse is given. *b*, The tension–voltage relationship obtained with the different pulses on the same fibres. Similar results were obtained with other fibres, although in the case of procaine the effect varied between different fibres. ●, Normal Ringer solution; ○, 5 mM procaine; ▲, 0.25 mM tetracaine.

fibres stained with Nile blue A. Procaine, and to a greater extent tetracaine, are thought to inhibit calcium release from the sarcoplasmic reticulum[10,11].

Figure 1 shows the tension–voltage relationships obtained with three different fibres in the absence of local anaesthetics, in the presence of 0.25 mM tetracaine, or in the presence of 5 mM procaine using voltage-clamp depolarising pulses. It can be observed that 0.25 mM tetracaine is a more effective inhibitor of tension development than 5 mM procaine. Furthermore, it is important that with tetracaine (>0.1 mM) tension was abolished in every fibre tested, whereas with procaine the effect varied considerably between fibres. This variability may account for the apparent discrepancy with the work of Thorens and Endo[12].

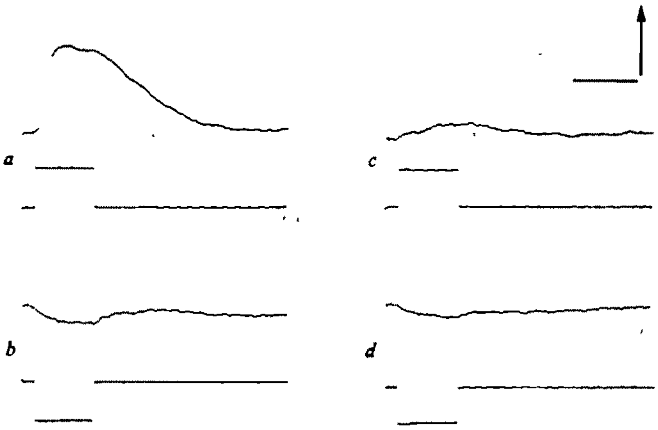

Fig. 2 Effects of tetracaine on the fluorescence signals in a stretched bundle of muscle fibres dissected from the semitendinosus muscle of *Calyptocefaleya gayi*, stained in Ringer plus 0.02 mg ml^{-1} Nile blue A (Allied Chemical). The chamber and optical arrangements were the same as previously described[4,6]. The bundle was stretched along a trough separated by Vaseline into a narrow central compartment (4 mm wide), where the bundle was illuminated, and two lateral compartments. Fibres in the bundle were depolarised by making the central compartment negative with respect to the lateral ones (positive currents) and hyperpolarised by reversing the polarity. To eliminate tension further and prevent regenerative action potentials, the compartments were filled with hypertonic 345 Tris Ringer solution (345 mM Tris-hydroxymethyl aminomethane-Cl, 1.8 mM $CaCl_2$ and 2.5 mM KCl). In each section, the upper trace shows the fluorescence signal and the lower trace the current pulse. In *a* and *b*, 345 Tris Ringer was used and in *c* and *d* 1 mM tetracaine was added to 345 Tris Ringer. *a*, *c* Correspond to depolarising current pulses and *b*, *d* to hyperpolarising current pulses. The magnitude of the arrow corresponds to a ratio of fluorescence change to basal fluorescence ($\Delta F/F$) equal to 2×10^{-3} for *a* and *b*, and 1.5×10^{-3} for *c* and *d*. The horizontal calibration bar corresponds to 100 ms. The excitation filter used in the optical record had a central wavelength of 625 nm and the collected light, at right angles, was filtered by a barrier filter that absorbed the excitation light (Schott RG-645). Temperature, 17 °C.

Figure 2 demonstrates the effect of tetracaine on the extrinsic fluorescence signal of muscle fibres stained with Nile blue. Fluorescence signals in response to depolarising (Fig. 2*a*) and hyperpolarising (Fig. 2*b*) current pulses indicate that, although of the same magnitude, the two pulses induce widely different fluorescence changes. The fluorescence change in response to the depolarising pulse is much the greater, suggesting the presence of a nonlinear membrane potential change. This potential change cannot be due to sodium-dependent regenerative responses, as the external solution was prepared without this cation. Figure 2*c*, *d* also shows that 1 mM tetracaine effectively abolishes the large component of the fluorescence change elicited by the depolarising pulses. The remaining signals behave more linearly for depolarising and hyperpolarising pulses, giving positive and negative fluorescence changes, respectively, with time courses similar to those recorded with Merocyanine 540 (ref. 4). The responses to hyperpolarising pulses are larger, probably due to increased membrane resistance in the presence of tetracaine.

Figure 3 shows the effect of procaine on the fluorescence activation curve. It is clear that in the absence of procaine, this

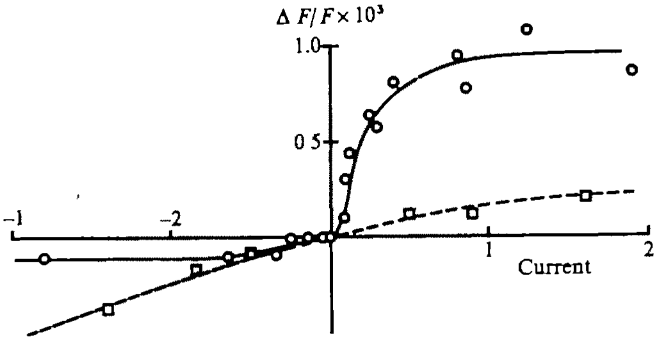

Fig. 3 Effect of procaine on the activation curve of the fluorescence signal. The points were obtained from an experiment in a bundle of fibres dissected from the semitendinosus muscle of *Calyptocefaleya gayi*, the procedure was the same as shown in Fig. 2 except that 10 mM procaine was used instead of tetracaine. The curve is a plot of $\Delta F/F$ against the current intensity of the depolarising and hyperpolarising pulses. The abscissa represents the size of the current pulse in arbitrary units. It must be stressed that the amount of current passed depends on the efficiency of the Vaseline seal between the three compartments (see legend to Fig. 2). The ordinates represent the peak values of the fluorescence signals. Circles correspond to experimental values obtained in 345 Tris Ringer and squares to values recorded in 345 Tris Ringer + 10 mM procaine. Temperature, 17 °C.

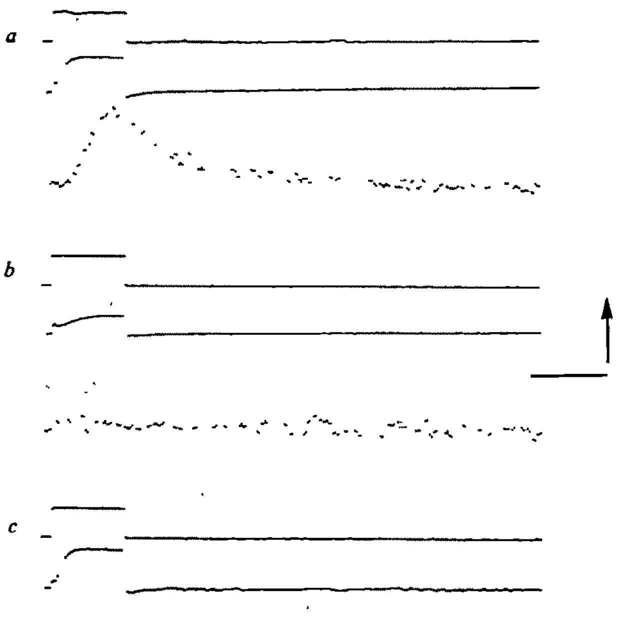

Fig. 4 Effect of tetracaine on the fluorescence signals and delayed rectification of voltage-clamped, cut single muscle fibres. The fibres were dissected from the semitendinosus muscle of *Rana catesbiana*. The techniques and optical arrangement used are described elsewhere[7] and are based on the work of Hille and Campbell[14]. Pool A, (see ref. 14) contained normal Ringer + 10^{-7} TTX. Pools C, B and E contained the following solution 120 mM K-aspartate; 5 mM ATP-Na_2; 1 mM $MgCl_2$; 2 mM EGTA-Tris; *p*H 7.2. In each section, the upper trace corresponds to voltage, the middle trace to the current, and the lower trace to fluorescence. *a*, Control records when the portion of the fibre in Pool A was immersed in Ringer + 10^{-7} M TTX. *b*, Records in Ringer + 10^{-7} M TTX + 1 mM tetracaine. *c*, Records after 30 min of recovery. The arrow corresponds to a $\Delta F/F$ of 7.2×10^{-3}, a voltage of 200 mV and a current of 13 µA. The horizontal calibration bar corresponds to 30 ms. Temperature, 20 °C.

curve has a steep S-shaped form reminiscent of the tension curve. On the other hand, in the presence of 10 mM procaine, the steep activation of the fluorescence for depolarising current pulses disappears, leaving only the more linear dependence probably arising at the surface and tubular membranes.

Finally, Fig. 4 shows the effect of 1 mM tetracaine on the fluorescence signal and on the membrane current of a single muscle fibre, voltage clamped, using the technique described by Hille and Campbell[14] but with a modified chamber which also allowed optical recording of fluorescence[6,7]. Figure 4*a* shows the voltage trace, membrane current and fluorescence trace for a fibre immersed in Ringers and 10^{-7} M tetrodotoxin (TTX). The fibre was clamped at about −100 mV and depolarised to 0 mV over 100 ms. It can be seen that tetracaine produced a complete blockage of the fluorescence signal. No linear component is observed, probably due to the small signal to noise ratio of the single fibre preparation. Figure 4*c* shows some recovery after 1 mM tetracaine was removed. Better recovery was observed when lower concentrations of tetracaine were used. The delayed current was blocked by the presence of tetracaine (Fig. 4*b*) and shows a good recovery on its removal (Fig. 4*c*). This current corresponds to the delayed K current whose kinetics are slowed and whose voltage dependence has been shown to be shifted by tetracaine[15]. Note that the recovery of the fluorescence signal does not parallel the recovery of this delayed current.

The results of these experiments show conclusively that procaine and tetracaine are effective in reducing or abolishing both tension development and the fluorescence signal in Nile blue A-stained muscle fibres. It has been postulated that this fluorescence signal is the optical manifestation of depolarisation of the membrane of the sarcoplasmic reticulum probably associated with calcium release[2]. Recent work carried out with local anaesthetics indicates that these agents are effective in blocking calcium release acting at the level of one of the last steps in the chain of events involved in excitation–contraction coupling. In fact, Almers and Best[11] have shown that tetracaine blocks contraction without affecting the charge movement; this indicates that calcium release can be inhibited one step further than the charge movement which is thought to link the electrical events of the transverse tubular membrane with those occurring at the level of the sarcoplasmic reticulum. The present experiments agree with this hypothesis and support the notion that the extrinsic fluorescence signal in Nile blue-stained muscle fibres may be derived from the sarcoplasmic reticulum membrane[2] and is associated with calcium release during contractile activation.

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Pelvic structure and nature of reproduction in Multituberculata

RECENTLY prepared skeletons of Mongolian Late Cretaceous multituberculate mammals permit complete reconstruction of the pelvic girdle for the first time; its structure suggests that multituberculates may have been viviparous. Most important for these studies is a specimen of *Kryptobaatar dashzevegi*[1] from the Djadokhta Formation (?late Santonian and/or ?early Campanian), in which both the left and right pelvic halves, including equipubic bones, were preserved undistorted and articulated with part of the vertebral column (Figs 1 and 2). Three of the pelves in the same collection, belonging to three different genera are less complete, but have the same structure as the *Kryptobaatar* pelvis. The previously known multituberculate pelvis of the Paleocene *Eucosmodon*[2,3] was incomplete and although correctly reconstructed did not show some important details, that are now described.

Hopson[4] argued that viviparity was one of the last elements in a syndrome of reproductive adaptations to have evolved in mammals. We do not know when viviparity developed in multituberculates, but it should be remembered that the Late Cretaceous multituberculates that have been studied are some 130 Myr younger that their presumable Triassic ancestors[*]. If they were indeed viviparous, such a mode of reproduction in multituberculates was developed independently from that seen in therian mammals.

The sexes of individuals represented by the fossil pelves are unknown. All four pelves are of the same morphological pattern and for the purpose of the description that follows I assume that at least one was female and/or that pelvic sexual dimorphism was not an obvious feature in multituberculates.

As pointed out by Granger and Simpson[2], multituberculate pelves differ from those of other known mammals in being extremely narrow. The left and right pubes and ischia form a very acute angle and appear V-shaped from behind instead of meeting at an angle of about 180° as in most other mammals. In *Kryptobaatar* the opposite pubes are directed steeply ventromedially and meet at an angle of about 40°; the ischia meet at 45° anteriorly and progressively open up to 80° at the ischial arc. The ventral edges of the opposite ischia and pubes are very strongly fused and together form a prominent ridge. The height of the ridge is about 1 mm in *Kryptobaatar* and its length along the pubes and ischia is 15.2 mm (the bone is missing below the obturator foramen, probably due to damage, and only here has the ridge not been preserved). The greatest length of the pelvis is 32 mm. The posterior part of the pelvis in known multituberculates is strongly bent upwards as seen from the side and the ventral ridge of the fused ischia continues far dorsally which means that the free posterior edges of the ischia are short and the ischial arc is very small. The free edges of the ischia are about 3.8 mm long, and the estimated greatest width of the ischial arc is about 4 mm. The length of the pelvic ridge and degree of fusion indicate that the pelvis was rigid and could not have spread ventrally during parturition.

The nature and dimensions of the pelvic girdle bear on the important question of the size of the offspring or egg in multituberculates and so on their mode of reproduction. The cross sectional area of the passage above the ischial arc depends on the position of the tail, which is high in *Kryptobaatar.* Granger and Simpson[3] suggested that *Eucosmodon* had a large iliac-sacral angle of 45° or more. This angle appears to be about 35° in *Kryptobaatar*, leaving a distance of about 1–1.5 mm between the base of the tail and the upper border of the iscial arc. Allowing ~0.2 mm thickness for the soft tissues lining the pelvis, I estimate that if the multituberculates were viviparous, the maximum width of the head in a newborn *Kryptobaatar* could be no more than 3.4 mm. This is about 0.13 the width of the mother's

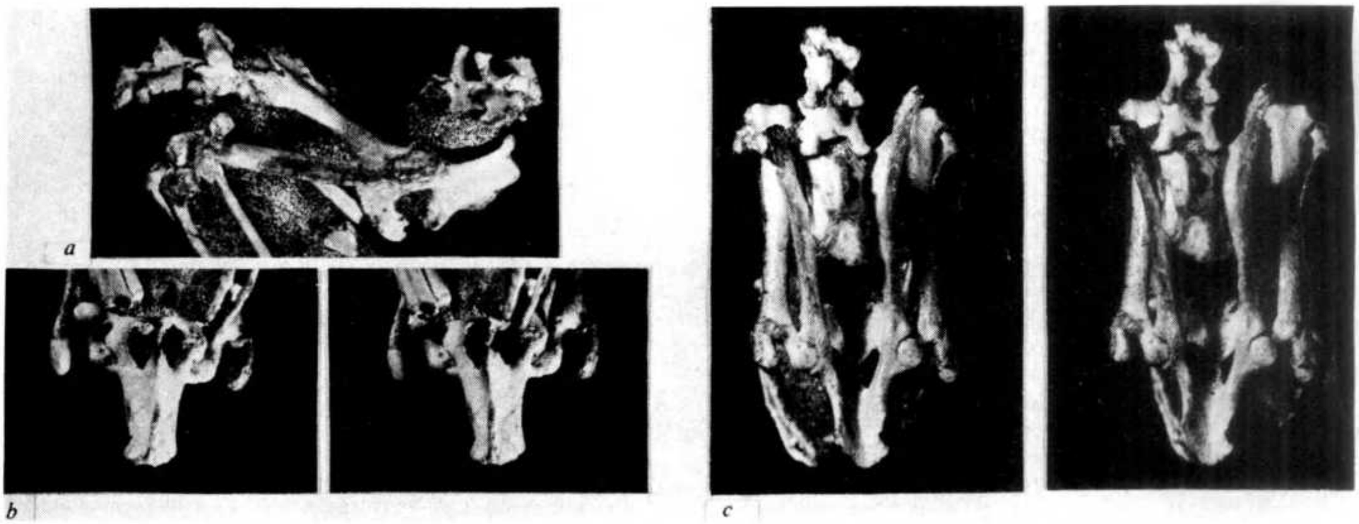

Fig. 1 *Kryptobaatar dashzevegi* pelvis. *a*, Left lateral view, photograph taken before final preparation; *b*, stereophotograph of the same specimen in ventral view, showing the ventral ridge; *c*, stereophotograph of the same specimen in dorsal view, the ischial tuber (present in *a*) was lost during preparation. ZPAL MgM-I/41, all ×1.5.

head compared with a ratio 0.33–0.4 in a rat and 0.45–0.5 in a mouse. There are no measurements available in the literature of the width of the head in newborn marsupials. But all marsupials weigh remarkably little at birth (all less than 1 g), even when the mothers are large[5].

As in *Kryptobaatar* the profile of the passage within the ischial arc is triangular, the space available for the passage of an egg of circular cross-section would be even less than 3.4 mm diameter. This would mean a smaller egg than any known cleidoic egg. In connection with the egg-laying habitat, the ischial arc in monotremes is wide and U-shaped[5] and relatively larger and structurally very different from the multituberculate ischial arc. I am therefore of the opinion that multituberculates were not oviparous, but viviparous with an extremely small neonate.

Apart from its relevance to reproductive habitat, the long immobile symphysis (which is relatively longer than in marsupials) and the well developed pelvic ridge may have a bearing to the mode of locomotion in *Kryptobaatar*. Some ungulates (for example *Antilocarpa* and *Tragulus*) have an immobile symphysis due to the presence of an unpaired interpubic bone and Wegner[7] argued that this is an adaptation in modern mammals associated with a capacity to jump. Perhaps multituberculates were also able to jump.

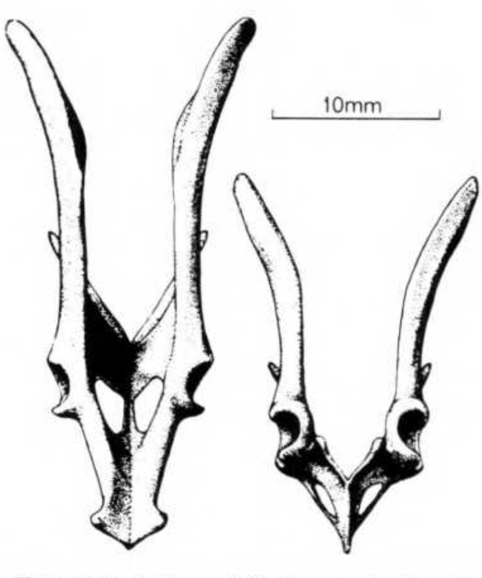

Fig. 2 *Kryptobaatar dashzevegi*. Right, reconstruction of pelvis in dorsal view. Left, reconstruction of the same in posterior view, showing size and shape of ischial arc.

Note added in proof: A. K. Lee (Monash Univ.) and L. Selwood (LaTrobe Univ.) have studied a marsupial *Antechinus stuarti* and found the mother's head width to be 15.4 mm, and the two newborn pouch young head widths were 1.6 mm and 1.5 mm, which is about 0.1 of the width of the mother's head (personal communication). This conforms with my estimate of 0.13 for multituberculates.

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Isolation of the human placenta receptor for epidermal growth factor-urogastrone

EPIDERMAL growth factor-urogastrone (EGF-URO), a single-chain polypeptide of molecular weight ~6,000, found both in the mouse[1-3] and in man[4-7], is both a potent stimulant of cell proliferation and an inhibitor of gastric acid secretion. Specific membrane receptors for EGF-URO can be detected in various tissues including human placenta[8-11]. Although the mouse and human polypeptide sequences differ at 16 positions, they can share the same receptor site in human tissues[3,12]. There has been considerable recent interest in EGF-URO and its receptor, not only because of the potent mitogenic and acid-inhibitory actions of the polypeptide, but also because of the association of viral[13], chemical[14] and spontaneous[15] cell transformation with a specific reduction in cell receptors for EGF-URO. It is also believed that

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EGF-URO and its receptor may be involved in the development, maintenance, and differentiation of tissues as diverse as the lens[16], the palate[17] and the keratin layer of skin[18]. In experiments aimed at the isolation of the membrane receptor for EGF-URO, we have observed that, unlike the receptor for insulin[19], but like the muscarinic receptor for acetylcholine[20], the EGF-URO receptor loses its ligand recognition property following solubilisation with detergents. We therefore developed an affinity-labelling technique using glutaraldehyde, that can covalently crosslink the receptor with ^{125}I-labelled EGF-URO before solubilisation[21]. Independently, Das and coworkers have developed a photoaffinity-labelling technique that can also be used to radiolabel the EGF-URO receptor before solubilisation[22]. Here we report the success of both our previously described glutaraldehyde crosslinking technique[21] (using ^{125}I-EGF-URO for receptor labelling) and of a newly developed photoaffinity-labelling technique (using a photolabile ^{125}I-EGF-URO crosslinking reagent) to identify the EGF-URO receptor in human placenta membranes and to effect the isolation of the affinity-labelled receptor by affinity chromatography using lectin and murine EGF-URO antibody–Agarose columns.

Table 1 Affinity chromatography of ^{125}I-photoaffinity-labelled EGF receptor

Lectin	Eluting sugar	Radioactivity (%) Unadsorbed	Sugar eluate	Sugar-urea eluate	Residual
WGA	*N*-Acetylglucosamine	94.8	4.4	0.7	0.1
Lens culinaris	α-Methylmannoside	95.8	3.6	0.4	0.2
Concanavalin A	α-Methylmannoside	93.0	3.0	2.5	1.4
Phytohaemagglutinin	*N*-Acetylgalactosamine	95.5	1.1	2.0	0.9
Ricinus communis	Galactose	99.7	0.2	0.0	0.0
Peanut Lectin	Galactose	99.8	0.1	—	0.1

The EGF-URO receptor of placenta membranes was labelled using the photoaffinity method as described in Fig. 1 After solubilisation with Nonidet P-40 and clarification by ultracentrifugation, aliquots of the labelled solubilised membranes were applied to 200 μl lectin–Sepharose 4B columns (5 mg lectin per ml beads) equilibrated with 0.1% Nonidet P-40, 0.2 M NaCl, and 50 mM Tris-HCl, *p*H 7.5. The columns were washed with 20–30 column volumes of buffer (flow through) and then eluted with an appropriate lectin-specific sugar at 0.2 M. The plant lectin columns were subsequently eluted with sugar-containing buffer, made 4 M in urea. Finally, the radioactivity remaining on the column was measured. The figures represent the percentage, relative to the total radioactivity applied, of the radioactivity in the unadsorbed fraction, in the fraction eluted with sugar, in the fraction eluted upon addition of urea, and the amount remaining on the column after the final elution (residual).

When placenta membranes were crosslinked covalently with ^{125}I-labelled EGF-URO, either by the glutaraldehyde method or by the photoaffinity technique, electrophoretic analysis of the solubilised membrane protein in polyacrylamide slab gels revealed an identical autoradiographic pattern (Fig. 1); two major components (bands I and II) of apparent molecular weights 180,000 and 160,000 could be detected. The relative amounts of the two components present seemed to vary somewhat from one preparation to another, as indicated by differences in the intensity of the autoradiographic patterns. When crosslinking was carried out in the presence of an excess of unlabelled EGF-URO, the labelling of bands I and II was virtually abolished, whereas that of other barely detectable components remained unchanged.

Previous data[15,21,23,24] suggested that the EGF-URO receptor is a glycoprotein. We observed that a variety of lectin–Agarose columns could adsorb selectively the major labelled components present in the crude solubilised membrane preparation (Table 1 and Fig. 1), enabling selective desorption with the appropriate lectin-specific sugar to take place. The wheat germ agglutinin (WGA)-agarose column proved to be the most effective, resulting in a 30-fold receptor purification using crude soluble preparations as starting material. Analysis of the specific eluate from the WGA column revealed the presence of a third minor radioactively-labelled component of apparent molecular weight 48,000.

The amino terminus of murine EGF-URO provides the only free primary amine and is the site of the coupling of the photoaffinity label. Because the remainder of the molecule is unchanged, we hypothesised that the anti-EGF-URO antibody would still recognise the EGF-URO moiety after crosslinking it to the putative receptor. The material eluted from the WGA-agarose column was therefore applied to an anti-EGF-URO antibody–Agarose column. Approximately 40% of the applied radioactivity was adsorbed to the column and more than 95% of the protein was unadsorbed. Elution of the antibody–Agarose column with 10% v/v formic acid, yielded the same components, detected autoradiographically, that had been adsorbed by the WGA–agarose column (Fig. 1, columns *f* and *h*). In the experiment depicted (columns *e*–*h*), the preparation of labelled receptor contained principally band II and a small proportion of band I; both bands I and II were adsorbed by the antibody affinity column. The amount of protein present in the eluate could not be detected (absorbance at 280 nm). When the same amount of WGA–agarose column eluate was applied to an agarose affinity column prepared with pre-immune rabbit serum, >95% of the radioactivity was unadsorbed and the characteristic autoradiographic pattern of the formic acid eluate previously observed with the antibody–agarose column was not detected. Similarly, when crude soluble membrane proteins were purified by WGA-agarose affinity chromatography, labelled with ^{125}I, and subsequently applied to an anti-EGF-URO antibody–agarose column and to pre-immune rabbit serum–agarose column, >95% of the radioactivity was unadsorbed. When the formic acid eluate was subjected to electrophoresis, bands in the region of I and II were not detected.

Because of the high specificity of the binding of EGF-URO to membrane-binding sites[8–12,16,22] and because of the correlation of this binding with the biological activity of EGF-URO in certain cell systems[8,10], it is reasonable to assume that binding components detected in this and previous studies represent the cellular receptor involved in EGF-URO action. It is of particular importance that the two independent methods of radiolabelling the receptor yield identically labelled components, as indicated by electrophoretic analysis. It is perhaps surprising, as previously discussed[21], that the glutaraldehyde–borohydride technique does not yield a wide spectrum of crosslinked components, but probably links ^{125}I-labelled human EGF-URO to its binding site in a relatively restricted membrane environment. Important is the fact that, using the conditions we describe, the photoaffinity label does not, following generation of the nitrene, alkylate a large number of protein constituents, as is observed in intact cells[22]. Because the two methods of radiolabelling the receptor are chemically quite dissimilar and

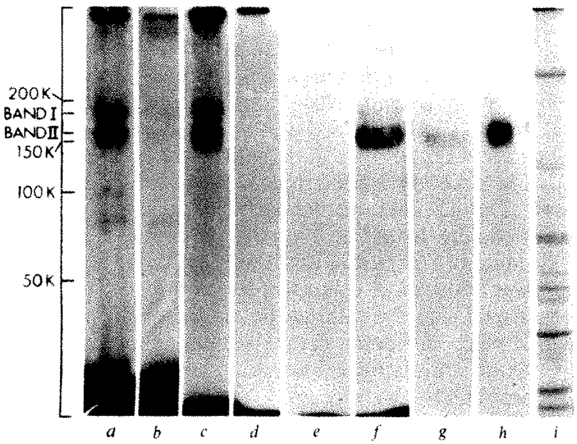

Fig. 1 Electrophoretic analysis and autoradiography of soluble protein from ^{125}I-EGF-URO affinity-labelled placenta membranes. Columns *a* and *b* represent the analysis of protein that was affinity-labelled either in the absence (*a*) or presence (*b*) of excess unlabelled mouse EGF-URO using the glutaraldehyde-borohydride method described previously[21]. Briefly, human placenta membranes (0.5 mg ml^{-1}), prepared by differential centrifugation, were equilibrated (40 min, 23 °C) with ^{125}I-labelled human EGF-URO, washed with ice-cold buffer, resuspended, crosslinked with the addition of glutaraldehyde (4.2 mM; 40 min, 4 °C), reduced with sodium borohydride (16.7 mM, *p*H 9.0, 30 min, 4 °C), solubilised (1% w/v Nonidet P-40, Particle Data Laboratories), clarified by centrifugation (200,000 *g*, 30 min) and the soluble supernatant analysed by polyacrylamide gel electrophoresis using 7.5% gels and the method of Laemmli[27]. Labelled protein was detected by autoradiography of fixed, dried gels. Columns *c* and *d* represent the analysis of soluble protein from membranes that were first photoaffinity-labelled either in the absence (*c*) or presence (*d*) of excess unlabelled mouse EGF-URO. The photoactive ^{125}I-EGF-URO derivative was prepared by first iodinating mouse EGF-URO, using the Iodogen (Pierce) procedure as described previously[29], with 5 μg of mouse EGF-URO and 2 to 3 mCi carrier-free ^{125}I in 200 μl of 0.3 M phosphate buffer, *p*H 7.6. After the addition of ^{125}I (40 s), the reaction mixture was transferred to a foil-covered vial containing 800 μl 0.1 M phosphate buffer, *p*H 7.6. Aliquots were removed for measurement of the amount of radioactivity present and of the incorporation of ^{125}I into EGF-URO. The volume was made up to 2 ml with buffer and *N*-succinimidyl-6-(4′-azido-2′-nitrophenylamino) hexanoate (SANAH; Pierce) was added in the dark (30 μl of 1 mg ml^{-1} in dimethylsulphoxide) to the stirred solution. The reaction (30 min, 24 °C) was terminated by the addition of 0.5 ml of 50 mM unbuffered lysine HCl. The product was stored in the dark at 4 °C and used without further purification. Photoaffinity-labelling was achieved by incubating the SANAH derivative of ^{125}I-labelled mouse EGF-URO (330 pmol) with membranes (40 ml of 1 mg membrane protein per ml in 0.1 M NaCl, 50 mM Tris HCl, *p*H 7.6) for 40 min at 23 °C in the dark and then activating the azide group by illumination (two 200 W incandescent bulbs at 10 cm) at 4 °C for 10 min. Labelled membranes were collected and washed twice by centrifugation (48,000 *g* for 20 min), solubilised (4 mg ml^{-1} in 0.5% Nonidet P-40) and the clarified extract (200,000 *g*, 30 min) analysed by gel electrophoresis with visualisation of labelled protein by autoradiography. Columns *e* and *f* represent the analysis of soluble photoaffinity-labelled protein which was applied to a 300 μl wheat germ agglutinin (WGA)-Sepharose 4B column (4 mg WGA ml^{-1} beads) prepared by the method of March *et al.*[28] and equilibrated with buffer (0.1% w/v Nonidet P-40, 0.2 M NaCl in 50 mM Tris HCl, *p*H 7.6). Column *e* represents the analysis of the unadsorbed fraction; column *f* represents the adsorbed material that was eluted from the WGA-Sepharose column with 0.2 M *N*-acetylglucosamine (NAG) in buffer. Columns *g* and *h* show the analysis of protein that was first specifically eluted with NAG from WGA-Sepharose and then applied to an anti-mouse EGF-URO antibody-Sepharose 4B column. Column *g* represents the unadsorbed fraction; *h* shows material that adsorbed to the column and was eluted with 10% v/v formic acid. Column *i* shows the protein pattern of untreated placenta membranes stained with Coomassie Blue. Bands I and II represent the major affinity-labelled proteins; the left hand scale shows the calibration of the gel according to the molecular weight markers *β*-galactosidase (130K), phosphorylase A (94K), bovine serum albumin (68K) and pyruvate kinase (54K).

yet yield identical results, we conclude that the risks of misinterpretations inherent in either crosslinking procedure have been minimised.

The apparent molecular weights of the two principal constituents detected in the present study are in excellent accord with our own previous data[21] and with the data of Das *et al.*[22], who detected a photoaffinity-labelled substituent of apparent molecular weight of 195,000 in mouse cells. We did not, however, detect, in the placenta membrane, the constituent of molecular weight ~100,000 which we observed in rat liver membranes using the glutaraldehyde-borohydride technique[21]. Detection of more than one receptor constituent cannot be readily explained. The most likely explanation, however, is that the two major constituents represent molecules that are closely related to one another either by a degradative (for example, receptor processing) or synthetic (precursor-product) process. These possibilities may explain the variation in the relative amounts of the two major constituents that we have observed from one membrane preparation to another. It is of interest in this regard that the affinity labelling of the *β*-adrenoreceptor also yields two principal constituents[25]. No components analogous to the 'processed' receptor constituents detected by Das *et al.*[26] in an intact cell system seem to be detected by our techniques in the placenta membrane preparation.

The present work demonstrates directly the glycoprotein nature of the EGF-URO receptor. It also demonstrates a novel method for purifying receptors utilising anti-ligand antibodies and provides a scheme for the large scale isolation of receptors which, like the EGF-URO receptor, seem to lose their ability to recognise ligand after solubilisation and thus are not amenable to purification by hormone-agarose affinity chromatographic procedures.

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The molecular nature of heat-labile enterotoxin (LT) of *Escherichia coli*

ALTHOUGH *Escherichia coli* is part of the natural flora of the mammalian gut, certain strains cause a cholera-like disease in humans and young farm animals[1,2]. These *E. coli* strains carry at least two plasmid-mediated determinants of virulence[3,4]. One class of plasmids, called Ent, codes for the synthesis of one or more enterotoxins[3]. These toxins disrupt the fluid balance in the gut resulting in a net efflux of fluid and electrolytes from the gut epithelial cells[1]. The heat-labile toxin (LT) and cholera toxin have been shown to be identical in their modes of action[5] and also to be immunologically similar[6,7]. Although cholera toxin has been purified and characterised[8], it has proved difficult to purify LT and it is not yet fully characterised. Molecular weight estimates for LT range from 20,000 to over 100,000 (refs. 9–11). We report here investigations of the molecular nature of LT carried out by studying an LT gene isolated using recombinant DNA technology[12]. Using *E. coli* minicells[13], the proteins encoded by the LT gene were identified and the structure of LT was found to be similar to that of cholera toxin.

Recently we reported the isolation of an LT gene on a 5.8×10^6-MW DNA fragment[12]. Using *in vitro* deletion techniques, this DNA fragment has been reduced in size to MW 1.2×10^6 and has been linked to the R-plasmid pBR313 (W.S. D., D. M. Gill and S.F., in preparation). This new plasmid, EWD299, encodes for LT as measured by the *Y*-1 adrenal cell tissue culture assay[14]. Proteins specified by the chimaeric plasmid were identified using minicells as an *in vivo* protein synthesising system. Minicells do not contain chromosomal DNA but may contain plasmid DNA molecules. The plasmids are transcribed and the mRNA is translated in minicells.

Plasmid-encoded proteins from minicells were radioisotopically labelled by incubating purified minicells with ^{35}S-methionine or ^{14}C-amino acid mix. The total minicell proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and the plasmid-specified proteins were identified by autoradiography. EWD299 was found to encode about 12 distinct proteins, (Fig. 1 track *a*). The LT gene-specified proteins were identified among these labelled proteins using an antiserum prepared against a crude preparation of LT and a *Staphylococcus aureus* strain containing protein A[15]. The protein A on the *S. aureus* binds antibodies without disrupting their active sites. Tertiary complexes of protein A, antibody and antigen can be formed and these aggregates can be separated from the unreacted antigens in the minicell lysate (see Fig. 1 for details). A 26,000-MW protein (Fig. 1, track *e*), was shown to bind nonspecifically to *S. aureus* cells and has been identified as β-lactamase[16]. Two other proteins of molecular weights 11,500 and 25,500 were found to be complexed in the presence of the anti-LT serum (Fig. 1 track *d*). These results may be interpreted in two ways. First, only one of the proteins is actually LT and the other protein was present in the extract used to raise the antiserum. On the other hand, LT may be a multimeric toxin composed of two distinct protein species.

Cholera toxin is a multimeric toxin composed of two structurally and immunologically distinct proteins[8]. Antisera can be prepared directed specifically against the adsorption protein, subunit B. This subunit binds the holotoxin to the target cell membrane, facilitating the activity of the A subunit, and has no toxic activity on its own. Antiserum prepared against subunit B was used in the *S. aureus* experiments and the results are shown in track *b*. Both the 11,500 and the 25,500-MW proteins were present, suggesting that LT is composed of at least two distinct proteins, one of which is immunologically related to the B subunit of cholera toxin. These data indicate that LT is present as a multimeric toxin intracellularly, as two proteins were isolated with an antiserum directed against a single protein. Track *c* contains a minicell preparation from an experiment using a holocholera toxin antiserum. Again, both the 11,500 and the 25,500-MW proteins are present.

Cholera toxin is composed of three polypeptides. The B subunit migrates in SDS-PAGE at an apparent MW of 11,000 (data not shown). Subunit A is composed of a 24,000-MW polypeptide linked by a disulphide bond to a 5,500-MW polypeptide[17]. The subunit structure we have found for LT is similar to that of cholera toxin but there are notable differences. The small subunit of LT, the 11,500-MW protein, is about 500 daltons larger than the B subunit of cholera toxin. Furthermore, there is no evidence of disulphide linkage in the 25,500-MW LT subunit. It has been shown that subunit A of cholera toxin has adenyl cyclase stimulating activity and a similar activity in crude LT preparations has been seen[20]. We also have enzymatic and genetic evidence that demonstrates an adenyl cyclase stimulating activity for the 25,500-MW protein (Dallas, Gill and Falkow, in preparation).

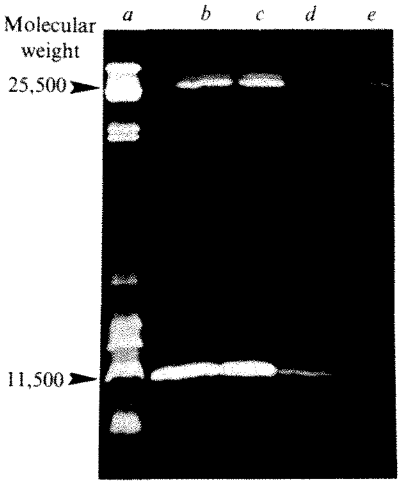

Fig. 1 Identification of the LT proteins. Minicells containing EWD299 were isolated and plasmid-encoded proteins were labelled by incubating the minicells with ^{35}S-methionine[16]. A minicell cytoplasmic fraction was prepared as follows. Labelled minicells were frozen and resuspended in phosphate buffered saline, 1% Triton X-100, 5 mM EDTA (PBSTE). Lysozyme was added to 100 μg ml^{-1} and the preparation was sonicated. The lysate was cleared at 35,000*g* for 30 minutes and the supernatant was used for the antisera experiments. An aliquot of antiserum was added to the supernatant and the solution was incubated at room temperature for 30 min. Formalised *S. aureus*[15] was added and the mixture was incubated for an additional 30 min. *S. aureus*–antibody–antigen complexes were isolated by pelleting the solution through 1 M sucrose in PBTSE (1,000*g*). The pellet was boiled for 3 min in final sample buffer (10% glycerol, 5% β-mercaptoethanol, 3% SDS and 0.0625 M Tris, *p*H 6.8). Aliquots were electrophoresed on 10–15% gradient SDS-polyacrylamide gels[19]. The gels were dried onto filter paper and autoradiographed for 3–14 d. Track *a*, total minicell lysate; track *b*, anti-cholera B subunit serum; track *c*, anti-cholera toxin serum; track *d*, anti-LT serum; track *e*, no anti-serum added.

The primary structure of cholera toxin subunit B is established[18]. It should be possible to determine the primary structure of the 11,500-MW LT protein by nucleotide sequencing and to compare the amino acid sequences of these two proteins. Analysis of the primary structure of the two large toxin proteins may give further information concerning the evolution of these two similar enterotoxins.

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Escherichia coli lac operator mRNA affects translation initiation of *β*-galactosidase mRNA

THE primary function of the start of a message (mRNA for a specific polypeptide) is to ensure the correct initiation of translation. Little is known about the factors determining the frequency of translation or indeed if its variability is important in the total expression of genes. Recently, an analysis was introduced that allows calculation of *in vivo* frequencies; it was shown that the *in vivo* initiation rates of the first and last messages of the *lac* mRNA differ by fivefold[1]. Different initiation frequencies could be effected, in part at least, by a sequence of three or more nucleotides about 8 to 15 residues upstream from the initiating AUG codon that is complementary to a purine-rich sequence at the end of the 16S ribosomal RNA[2,3]. Four such complementary nucleotides are residues 8–11 before the AUG start of the *β*-galactosidase (EC 3.2.1.23, *β*Gase) message. Mutants for this sequence are not available, but there is a whole series for a more distant region—22 to 34 nucleotides before the AUG: the *lac* O^c mutants. Here we show that certain of these mutations affect the rate of translation as well as the stability of this message.

Lac O^c mutants are defective for binding repressor protein to the *lac* operator. The more than 800 O^c mutants of Smith and Sadler[4] could be grouped into seven classes defined by *P* values (ratio of basal to induced *β*Gase levels). So far, representatives of each class have been identified as single-base change mutants[5] which map in the initial sequences of the *lac* mRNA[6] (Fig. 1). This primary effect on the repressor–operator binding does not explain other changes in *β*Gase expression. The amount of fully induced *β*Gase (zero repression) differs between strains, with some accumulating more enzyme, and some less, than does the O$^+$ parent[4,7]. Thus, it seemed possible that the translation initiation frequency was affected by these mutations.

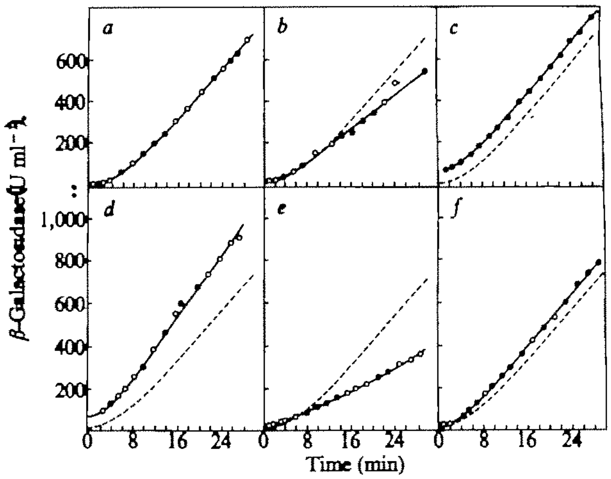
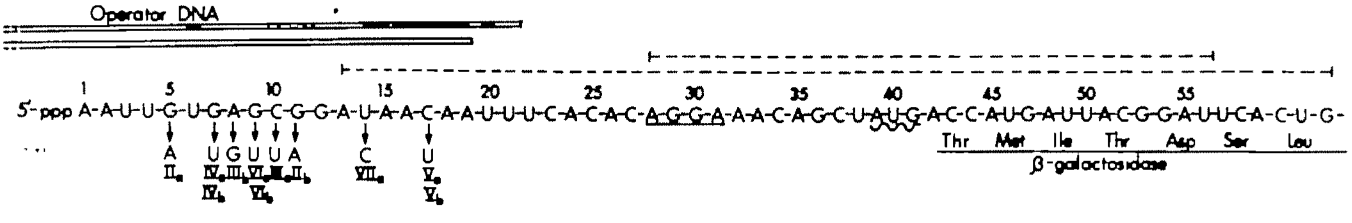


Fig. 2 The induction of *β*Gase in various *E. coli* HfrC, *proC*, *metB*, *strS lac* O^c mutants and O$^+$ parent strain described by Sadler and Smith[4]. Bacteria were grown at 37 °C in M9 inorganic salts[26] with methionine (to 50 μg ml^{-1}), proline (to 50 μg ml^{-1}), casein hydrolysate (to 0.4%) plus 0.2% glycerol and were induced at zero time by the addition of isopropyl-*β*-D-thiogalactoside (IPTG) (to 0.5 mM) after addition of cyclic AMP (to 2 mM) at −2 min to minimise catabolite repression. Samples were removed at the indicated times and brought to 0 °C, and metabolism further inhibited by addition of chloramphenicol (to 50 μg ml^{-1}) plus sodium azide (to 10 mM). The rate of *β*Gase synthesis was calculated after 15 min of induction when it had reached its maximum. At this time there were 2.6×10^8 viable bacteria per ml with 32 μg RNA per ml. *a*, Parent strain O$^+$, *b*, strain IIIa, *c*, strain IIb; *d*, strain IIa; *e*, strain IIIb; *f*, strain Vb. The synthesis of *β*Gase in the parent strain (*a*) is reproduced for comparison as a dashed line in each panel.

Rates of *β*Gase synthesis during full induction are required to estimate translation-initiation frequencies. Of six representative strains shown in Fig. 2, IIb and Vb are similar to the parent, IIa gives stronger induction, and IIIa and IIIb give weaker. The ratios of induction rates seen here between strains are similar to the ratios of fully induced enzyme levels observed earlier[4,7]. The turnover number for *β*Gase is ~400,000 U mg^{-1} (refs 1, 8) and its tetrameric molecular weight is 465,000 (ref. 9). With these values and the viable cell count determined at the time of induction measurement, the synthesis of *β*Gase monomers per s


Operator DNA
1
5
10
15
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5′-ppp A-A-U-U-G-U-G-A-G-C-G-G-A-U-A-A-C-A-A-U-U-U-C-A-C-A-C-A-G-G-A-A-A-C-A-G-C-U-A-U-G-A-C-C-A-U-G-A-U-U-A-C-G-G-A-U-U-C-A-C-U-G-
A
U G U U A
C
U
II$_a$
IV$_a$ III$_b$ VI$_a$ II$_a$ II$_b$
IV$_b$ VI$_b$
VII$_a$
V$_a$
V$_b$
Thr Met Ile Thr Asp Ser Leu
β-galactosidase

Fig. 1. The nucleotide sequence of the start of the *lac* mRNA as determined by Maizels[6]. The base substitutions expected in the mRNA from the changes in DNA sequence of the various O^c strains, determined by Gilbert *et al.*[5] are shown. The wavy line designates the initiation codon for the start of the *β*Gase polypeptide and the underlined AGGA could be a potential recognition site for UCCU at positions 3–6 from the 3′ end of the 16S rRNA. The sequences derived from the right side of the operator DNA, as defined by protection from repressor binding[24], as well as the shorter length defined from the minimal stretch that can form stable repressor complex[25], are shown by the horizontal bars. The dashed lines show the residues protected from RNase degradation by a ribosome initiation complex. The longer segment is the maximum length protected against RNase T1 and the shorter is the minimum length protected against RNase A[13]

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cell is calculated in Table 1, column 2. The parent HfrC strain produces 19 monomers per s cell, strain IIa 25, and at the other extreme, IIIb only nine.

Three parameters of gene expression combine to give these final rates of enzyme synthesis at any instant. (1) The number of message molecules producing finished polypeptides, (2) the rate of translation initiation, and (3) the rate of ribosome movement. The first parameter is the most difficult to measure. It is derived from the total z mRNA mass which is given most accurately by dividing its rate of synthesis (S) by its decay constant (k) (refs 1, 10) and then converting mass to numbers of completed molecules[1].

The rate of z message synthesis is derived from the fraction of 30 s pulse-labelled RNA that is z-specific in these fully induced cells (about 1.5% after correction[1] for hybridisation efficiency). As these strains double every 50 min, they contain 1.7 copies of the *lac* operon per cell[1]; this gives about 2.5 s between transcription initiations and 100 nucleotides between RNA polymerases (column 3). These mutations do not affect the rate of transcription initiation. None of the strains show functional or mass decay slower than that of the parent ($t_{1/2} = 1.5$ min) (Fig. 3), but decay in IIa, IIIa, Va, Vb and VIa is significantly faster ($t_{1/2} < 1.2$ min) (column 4).

After subtracting the mass of nascent z mRNA, the number of completed molecules can be calculated (column 5, and Table 1 legend). Combined with the rate of ribosome movement (about 40 nucleotides per s per ribosome from the 80 s induction lag[11,12]) the translation initiation rate (column 6) and resultant density of ribosomes can be calculated. The loading frequency of the parent averages 4.9 s between initiations to give 176 nucleotides between ribosomes. Six O° strains differ: IIa, Va, Vb and VIa average only 3 s between translation initiations (110 nucleotides between ribosomes), whereas IIIb and IVb initiate significantly more slowly (8 s between initiations for strain IIIb to give 288 nucleotides between ribosomes.) There is a threefold range of initiation rates between these strains.

Mutations in the fastest-loading strains are not clustered; IIa and Va/Vb are 12 residues apart, and in fact, define the outer boundaries of the set. Conversely, the two mutations that give slower-loading are adjacent. All of these mutations lie outside the RNA region protected from RNase A digestion by the initiating ribosome[13] (Fig. 1). The IIa mutation is located about the diameter of a ribosome from the AUG start (assuming 4 Å per residue).

A possible secondary structure of the operator RNA can be considered (Fig. 4). Its free energy can be calculated from studies with synthetic polyribotides[14–17]. The favourable free energy from base pair stacking is about −8.6 kcal mol^{-1} at 25 °C, and the unfavourable energy from the constraints in closing the loop[14] would be about +7 kcal mol^{-1} (for a 9-base loop closed by AU) to give a net −1.6 kcal mol^{-1} at 25 °C. This structure should 'melt' at >25 °C; the T_m can be estimated from the enthalpy and entropy values that were also measured for the model polymers. Using values from two laboratories[14,15] yields a T_m of 45 °C or 58 °C. Note that the T_m of a monomeric duplex (with hairpin loop of this size) is much higher than is the T_m of the same duplex formed from two separate strands (see Fig. 4 in ref. 14). While the melting profile may be quite broad, it seems likely that most of the molecules are in this configuration at 37 °C.

What is striking is that the fast-loading strains IIa, Va, Vb give very similar decay and loading values and share the common feature of disrupting the single GC bond in the stem (Fig. 4) to generate a stem whose energy is now limited to the lower four AU pairs (−3.6 kcal) and has considerably less energy than the +8 kcal in the 11-base loop. If the secondary structure were a hindrance to ribosome loading, then such base changes would

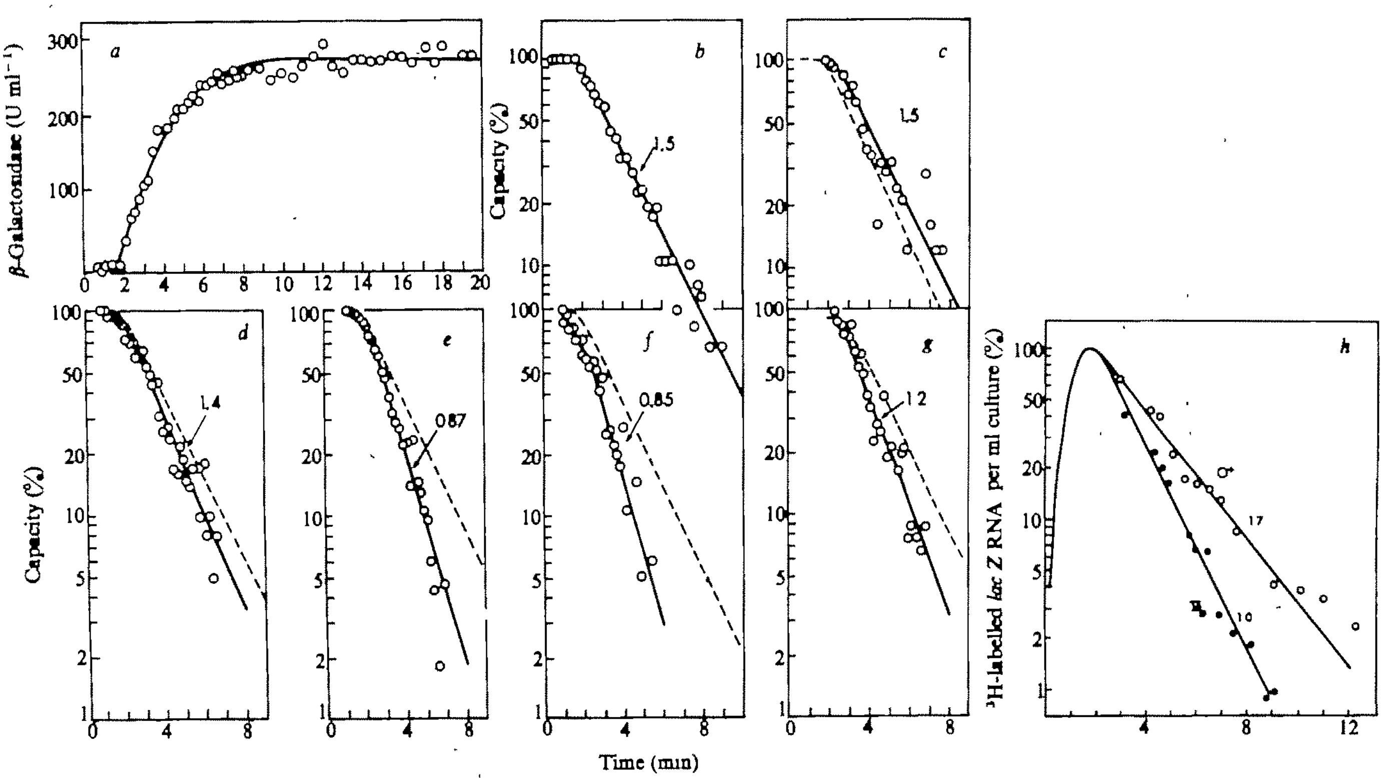

Fig. 3 Functional and mass decay of βGase message in various *E. coli lac* O° mutants and the O$^+$ parent. Cultures were induced at zero time and sampled as described in Fig. 2 except that rifampicin (to 300 μg ml^{-1}) was added at +30 s to inhibit further transcription initiations. *a*, The burst of βGase from parent strain O$^+$; *b*, the loss of capacity to synthesise enzyme[27] from the data in (*a*). The remaining panels only show this latter variable for each strain induced and sampled in the same way. *c*, Strain IIb; *d*, strain IIIb; *e*, strain Vb; *f*, strain IIIa; *g*, strain IIa. The half lives (min) are shown and the curve of the parent strain (*b*) is reproduced for comparison in each panel. Panel (*h*) shows the mass decay of βGase message in strain Vb and parent O$^+$ strain; [5,6-^{3}H]uridine (to 33 μl ml^{-1} and 2 nmol ml^{-1}) was added at −30 s and rifampicin as above. RNA extracted from each sample was reacted with denatured λp*lac*5 DNA to form hybrid and the hybrid fraction times the amount of ^{3}H-RNA per ml gives the relative amount of *lac z* message shown[28]. The half lives (in min) are shown.

Table 1 Expression of β-galactosidase gene in operator constitutive mutants of *E coli*

1 Strain	2 βGase synthesis monomers per cell per s*	3 Nucleotides per RNA polymerase	4 mRNA half-life(s) (ln 2/*k*)	5 Completed mRNA molecules per cell†	6 Time between translation initiations (s)‡
O$^+$	19	97	90	90	4.9
IIa	25	113	69	68	2.6
IIb	19	97	90	90	4.8
IIIa	12	90	54	63	5.1
IIIb	9	117	84	70	8.0
IVa	24	90	84	92	3 9
IVb	12	101	75	85	6.8
Va	23	95	69	81	3.3
Vb	19	84	57	67	3.4
VIa	20	97	63	70	3.2
VIb	15	105	78	86	5.3
VIIa	16	115	72	71	4.2

Exponentially growing bacteria were induced by addition of cyclic AMP (to 2 mM) and IPTG (to 0.5 mM) and rates of enzyme and message synthesis measured between 12 and 20 min after IPTG addition At 15 min there were 2.6×10^8 cells per ml with 32 μg RNA per ml. Divide amounts per cell by 1.66 to give amounts per chromosome. All values refer to the βGase gene, mRNA or enzyme

* There are four monomers per βGase to give 4.8 molecules of βGase per s per cell in strain O$^+$.

† The total mass of *lac z* mRNA/cell is made of: (*a*) completed *z* molecules that are not decaying, (*b*) completed *z* molecules in various stages of decay, and (*c*) incomplete or nascent molecules (some are also decaying), still on DNA and thus not releasing finished polypeptides at that instant. The mass of the nascent class can be estimated from *S* and subtraction from the total mass gives the mass of functioning molecules. The distribution between decaying and non-decaying species is derived from the 5′ to 3′ nature of the decay process[1] and leads to the actual numbers of functioning molecules per cell (column 5) (see ref. 1) The total *lac* mRNA per cell varies from 1.5×10^{-10} μg (three strains including O$^+$) to 1.0×10^{-10} μg (four strains) There are 52 nascent molecules per O$^+$ cell, 50 complete and 40 completed but decaying The number of completed molecules that are not decaying is the most variable with the faster-decaying strains (IIa, IIIa, Va, Vb, VIa and VIIa) containing only about 28 molecules per cell.

‡ All experiments were performed on three different cultures of each strain. The experimental values (*S*, *k*, and induction rate) represent averages with maximum deviations of ±10% each. If the experimental values for a strain were all in error by 10% 'in the same direction', the final computer-derived initiation frequency (column 6) would be in error 30% in that direction— insufficient to account for the 300% range of values.

decrease the probability of its existence and thus increase the frequency of successful collisions.

The slower initiation rates of strains IIIb and IVb might also reflect the presence of the secondary structure of Fig. 4. First, note that IVb would generate two more base pairs and a five-membered loop to increase the structural stability to $\Delta G = -5.1$ kcal mol^{-1} at 25 °C from -1.6 kcal mol^{-1}. However, IIIb would not affect the structural stability and suggests another possible explanation. Independent studies in this laboratory have shown an involvement of RNase III activity in translation initiation of βGase. βGase mRNA from strain AB301-105 (ref. 18) is much less competent to form *in vitro* initiation complexes than is βGase mRNA from the *rnc*$^+$ parent[19]. The RNase III recognition sequence has been defined in detail in four of six T7 RNA sites[20,21], and the four are similar: cleavage between U and G and release of terminal fragments UUAU$_{OH}$ and pGAU. Cleavage of *lac* RNA between the residues U and G at positions 6 and 7 would liberate UUGU$_{OH}$ plus pGAG. As indicated above, opening the loop would lower the T_m of the duplex. If this is a site for RNase III cleavage, then IIIb and possibly IVb, might load ribosomes more slowly because the enzyme could no longer disrupt the structure. The hypothesis is being tested by *in vitro* experiments.

Strains VIb, IIIa, IIb and VIIa give the parental O$^+$ initiation frequency, and they all map in the upper part of the loop structure and should not alter it. Thus, 9 of the 11 characterised *lac* O^c mutants give expression that is consistent with the structure shown in Fig. 4. Strains VIa and IVa give inconsistent results. It is possible that they were assigned incorrectly or contain secondary mutations in either *lac* DNA or the genetic

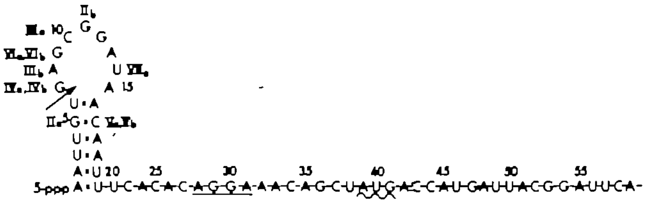

Fig. 4 A possible configuration of the *lac* mRNA[6]. The AGGA and AUG are designated as in Fig. 1. The arrow indicates a putative RNase III target.

background. In strains IVa and VIa expression of the distal *lac* galactoside acetyltransferase (GATase) message and βGase message change in parallel, suggesting some nonspecific effect, perhaps on all messages. In contrast, for example, the extremely fast βGase message decay in strain Vb is not paralleled by faster than normal (O$^+$) decay of GATase message (data not shown).

Thus, the major observation presented here is that the RNA region more than 21 residues before an AUG start is important for translation initiation as well as message stability. These two processes show no obvious correlation here (Table 1); however, other studies suggest a causal relationship between decay and loading frequency[1,22], perhaps obscured in these base change mutants because the vulnerability of the specific decay target must also be a function of the primary base sequence itself. A role of RNA secondary structure in initiation has been suggested, not only in the well known RNA phage translation, but in recent speculation concerning internal initiation sites of the *lac i* mRNA[23] which have potentially base-paired regions proximal to them. However, this is the first report to relate mutations in a potential secondary structure to measured changes (threefold range) in initiation frequencies.

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Double-stranded DNA stereoselectively binds benzo(*a*)pyrene diol epoxides

BEFORE it can exert its mutagenic and carcinogenic activities[1,2] benzo(*a*)pyrene (BP) requires metabolic activation and it is widely considered[3–5] that the initiating event in these processes is covalent binding to DNA. The predominant form of the activated hydrocarbon is a diol epoxide[6–11], of which four possible isomers are (+) and (−) enantiomers of 7β,8α-dihydroxy-9α-10α-epoxy-7,8,9,10-tetrahydro-BP (*anti*-BP diol epoxide) and (+) and (−) enantiomers of 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydro-BP (*syn*-BP diol epoxide). Enzymatic formation of the (+) *anti* and (+) *syn* configurations have been reported elsewhere[12,13]. We have shown that the racemic (±) *anti*-BP diol epoxide forms two stable covalent adducts each with the exocyclic amino groups of deoxyguanosine, deoxyadenosine and probably deoxycytidine[14]. We report here that we have now resolved the enantiomers of (±) *anti*-BP diol epoxide and reacted the optically pure hydrocarbons with DNA. Each enantiomer reacts with the exocyclic amino group of deoxyguanosine to form a pair of diasteriomers in a ratio which depends on the secondary structure of the nucleic acid. The (+) enantiomer reacts preferentially or asymmetrically with deoxyguanosine in double stranded DNA but the two enantiomers react equally with the same position in single-stranded DNA. The basis for the stereoselective binding is unknown but may involve formation of sterically orientated physical complexes before covalent attachment of the hydrocarbon to DNA. The mode of binding in the deoxyadenosine sites, on the other hand, must be different, as the (+) and (−) enantiomers react equally in either single- or double-stranded DNA.

The (±)BP diol epoxide was synthesised as described elsewhere[15,16]. ^{3}H-(±)*anti*-BP diol epoxide was synthesised by an adaptation of the method used by McCaustland *et al.*[17]. The (+) and (−) enantiomers of *trans*-7,8-dihydroxy-7,8-dihydro-BP were resolved by published methods[18] and converted to (−) and (+) *anti*-BP diol epoxides, respectively. Either racemic (±) *anti*-BP diol epoxide or the optically pure (>96%) (+) or (−) enantiomers were reacted with calf thymus or ΦX174 DNA. After removal of unreacted hydrocarbon the DNA was enzymatically hydrolysed to hydrocarbon–deoxynucleosides and the stable covalent adducts isolated by Sephadex LH-20 column chromatography. The adducts were then separated by reverse-phase high-pressure liquid chromatography (HPLC). Of the stable covalent adducts formed, 95% were recovered as determined by radioactivity measurements. Figure 1*a* shows the HPLC profile obtained by reacting racemic (±) *anti*-BP diol epoxide with native calf thymus DNA after hydrolysis and Sephadex LH-20 column chromatography.

We have previously identified adducts numbered 1–5, 7 and 8 (ref. 14). Peak number 1 was a tetraol hydrolysis product of the *anti*-BP diol epoxide, whereas adducts 2 and 4 represented deoxycytidine-derived products. The contribution of peak 4 to the total adduct formation was variable as it co-chromatographed with one of the tetraol hydrolysis products of the diol epoxide. Adducts 3 and 5 were reaction products with deoxyguanosine, and numbers 7, 8 and 9 were deoxyadenosine products. With native calf thymus DNA the deoxyguanosine adducts represented 90% of those formed. However, the distribution of adducts obtained depended on the reaction conditions used and was primarily a function of DNA secondary structure, that is, the more denatured the polymer the higher the proportion of adenine adducts obtained.

We have previously reported[14] that the pair of deoxyguanosine adducts (peaks 3 and 5) obtained from reaction of racemic (±) *anti*-BP diol epoxide with native calf thymus DNA were probably diastereomers, as their high resolution mass spectra were identical and their circular dichroism spectra were of similar shape but inverted. We have now demonstrated this result for both the deoxyguanosine and deoxyadenosine products by resolving the enantiomeric diol epoxides and carrying out adduct formation individually with DNA. The results of these reactions are shown in Fig. 1*b*, *c*. These profiles indicate that the (+) enantiomer reacted to form deoxyguanosine adduct 5 and deoxyadenosine adducts 7 and 9, whereas the (−) enantiomer resulted in deoxyguanosine adducts 3 and 6 and deoxyadenosine adduct 8. Peaks 6 and 9, although not previously

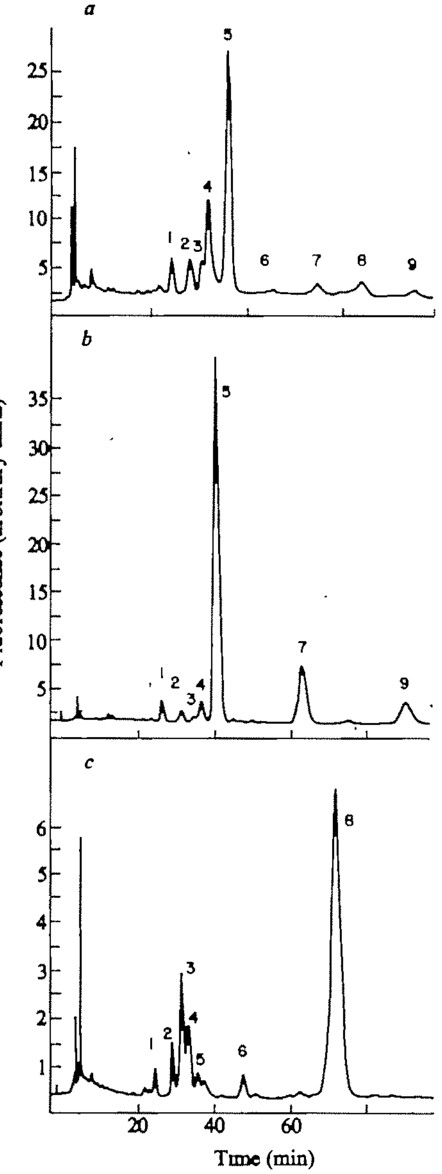

Fig. 1 Tritium-labelled racemic (±) *anti*-BP diol epoxide or the resolved enantiomers were incubated with 1 mg deproteinised native calf thymus DNA at an initial concentration of 1 hydrocarbon per 250 nucleotides in 0.01 M sodium phosphate, *p*H 7.5, for 1 h at 37 °C. Unbound hydrocarbon was removed by ethyl acetate extraction and repeated isopropanol precipitation of the DNA until zero time blanks contained <3% of the tritium of incubated samples. Routinely, incubated samples resulted in a modified DNA of 1 hydrocarbon per 8,000 base pairs The diol epoxide-treated DNA was converted to hydrocarbon–deoxynucleosides and deoxynucleosides by treatment with DNase II, spleen phosphodiesterase and alkaline phosphatase. The hydrolysed sample was applied to a Sephadex LH-20 column and the free deoxynucleosides and enzymes eluted with water. The hydrocarbon–deoxynucleosides were quantitatively recovered with methanol and analysed by HPLC[14]. The elution profiles represent DNA reacted with: *a*, racemic (±) *anti*-BP diol epoxide; *b*, (+) *anti*-BP diol epoxide; *c*, (−) *anti*-BP diol epoxide. The identity of adducts 1–9 are discussed in the text.

identified, correspond to deoxyguanosine and deoxyadenosine adducts, respectively, as indicated by field desorption mass spectrometry[19].

Reaction of nucleophiles with *anti*-BP diol epoxide has been shown to occur exclusively at the benzylic C-10 position, with a preference for *trans* addition[20-22]. It is possible, however, that DNA secondary structure could influence the stereochemistry of the epoxide ring opening to give *cis* addition products as well. As the D-deoxyribose moiety of each nucleoside unit within the DNA polymer contains an asymmetrical centre at C-1, the reaction of a racemic mixture of (±)BP diol epoxide would be expected to yield a maximum of four diastereomeric products per alkylation site ((+) diol epoxide per D-deoxyribose, each with *cis* or *trans* addition at C-10 of the hydrocarbon). Reaction of the metabolically produced (+) *anti*- and (+) *syn*-BP diol epoxide[12,13] with the exocyclic amino groups of guanine, adenine and cytosine in DNA would result in 12 possible diastereomeric covalent adducts. Recent indirect evidence[23] suggests that the (−) *anti* and (−) *syn* diol epoxides may also be made *in vivo*, and if this is the case then there would be 24 possible products by reaction of the four hydrocarbons with the three exocyclic amino groups in DNA. We obtained two resolvable deoxyadenosine diastereomers by reaction of the (+) *anti*-BP diol epoxide with DNA (adducts 7 and 9), which were probably the result of *cis* and *trans* addition of the adenine exocyclic amino group. Reaction of the (−) diol epoxide yielded peak 8 which by recycle chromatography consisted of two partially resolvable components. Each had virtually identical mass spectra[19], indicating that they were deoxyadenosine exocyclic amino adducts. Thus, the (−) enantiomer reacted with deoxyadenosine to give two adducts, in agreement with the above predictions.

Three hydrocarbon–deoxyguanosine adducts were observed in the reaction of racemic (±) *anti*-BP diol epoxide with DNA—peaks 3, 5 and 6. Peaks 3 and 5 correspond to the major and, presumably, *trans* addition products of (−) and (+) diol epoxide with N^2-deoxyguanosine, respectively. Peak 6, derived from the (−) enantiomer, was a relatively minor component and may correspond to either an exocyclic amino *cis* addition product or to a different guanine adduct with alkylation at some other site. For deoxycytidine only two adducts (peaks 2 and 4) were observed. The relative contributions of (+) and (−) *anti*-BP diol epoxide to these two peaks was difficult to assess, as peak 4 also contained varying amounts of a tetraol hydrolysis product.

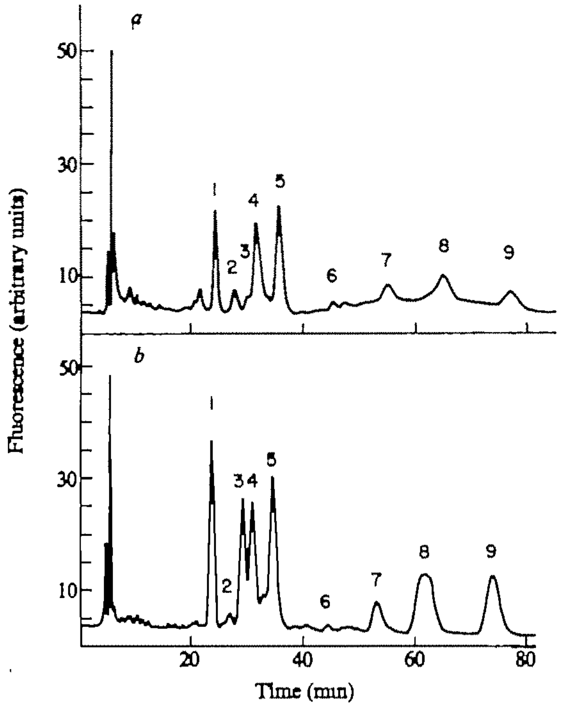

Fig. 2 Racemic (±) *anti*-BP diol epoxide was reacted with: *a*, double-stranded (replicative) ΦX174 DNA; *b*, single-stranded (viral) ΦX174 DNA. Adducts were formed and isolated as described in Fig. 1. The ratio of deoxyguanosine diastereomers derived from the (+) and (−) hydrocarbon is 20:1 for double-stranded DNA (the ratio of peaks 5 to 3, panel *a*), and 20:17 for the single-stranded polymer (the ratio of peaks 5 to 3, panel *b*). See text for discussion.

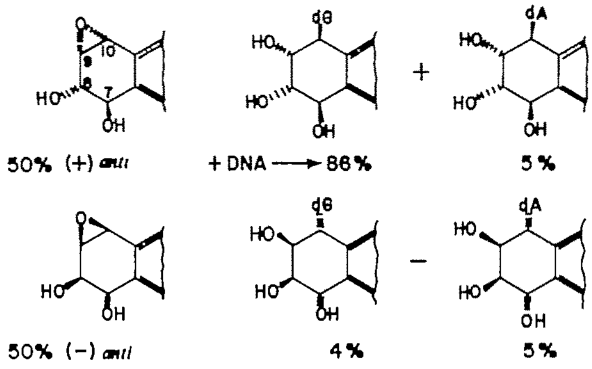

Fig. 3 The structure and per cent distribution of the principal guanine and adenine *trans*-addition products obtained by reacting racemic (±) *anti*-BP diol epoxide with native calf thymus DNA. The distribution also includes any *cis*-addition products (see text).

Figure 3 shows the structures of the *trans*-addition adducts and their per cent distribution obtained by reaction of racemic (±) *anti*-BP diol epoxide and native calf thymus DNA. (These percentages also represent any *cis*-addition products.) With the identification of adduct 9, we found that reaction of racemic (±) *anti* diol epoxide with DNA resulted in nearly equal distribution of deoxyadenosine diastereomer products, that is, adducts 7 and 9 from the (+) enantiomer of the hydrocarbon were about equal to adduct(s) 8 from the (−) enantiomer. By contrast, deoxyguanosine in DNA reacted stereoselectively with racemic *anti*-BP diol epoxide, that is, the ratio of adduct 5 derived from the (+) hydrocarbon to that of adduct 3 from the (−) enantiomer was 20:1. The same ratio of diastereomers was obtained by reaction of the optically resolved hydrocarbons and native calf thymus DNA (Fig. 1*b*, *c*). We initially postulated that the stereoselective binding could be a result of the hydrocarbons interacting asymmetrically with the secondary structure of DNA[14]. This postulate was tested by reacting racemic (±) *anti*-BP diol epoxide with double (Fig. 2*a*) and single (Fig. 2*b*) stranded ΦX174 DNA. The same distribution of stereoselective guanine adducts was obtained with native calf thymus DNA (Fig. 1*a*) and double-stranded ΦX174 DNA (Fig. 2*a*). Reaction of racemic (±) *anti*-BP diol epoxide with single-stranded ΦX174, however, resulted in nearly equal distribution of hydrocarbon-deoxyguanosine diastereomers (peaks 3 and 5). In addition, reaction with the single-stranded polydeoxynucleotide resulted in a significantly increased proportion of deoxyadenosine adducts. These same results were obtained with native and heat- or base-denatured calf thymus DNA. The ratio of deoxyguanosine diastereomers in the single-stranded calf thymus DNAs was approximately 1:1, and the proportion of deoxyadenosine adducts was 40% compared to 10% in the double-stranded polydeoxynucleotide.

These results demonstrated that the asymmetrical binding of the two enantiomeric *anti*-BP diol epoxides to the exocyclic amino group of guanine in double-stranded DNA was dependent on the secondary structure of the polymer. One possible mechanism for the ability of DNA secondary structure to distinguish two enantiomeric molecules is by stereoselective physical interactions (possibly intercalation) before covalent binding. Intercalation or some other physical interaction may readily occur with the (+) enantiomer but not with the mirror image hydrocarbon. Alternatively, the noncovalent interactions could 'fix' the hydrocarbon so that only one configuration is in a favourable position to react with the exocyclic amino group deoxyguanosine. The following observations suggest that intercalation or some physical association between the BP diol epoxide and DNA take place before covalent binding: (1) as with intercalation of BP[24] or actinomycin[25] into DNA, binding of the diol epoxide occurs with a preference for guanine sites, and (2) reaction with denatured DNA 'randomises' adduct formation (loss of stereoselectivity and more adenine binding). We propose such an intercalation model to account for the stereoselective interactions between BP diol epoxide and DNA. The lack of any stereoselectivity in the adenine covalent binding sites with either double- or single-stranded DNA suggests that the reaction mechanism may be different from that of guanine binding. In the case of adenine the hydrocarbon may react without any physical interactions modulating the ratio of diastereomers obtained.

A correlation between DNA binding and carcinogenic or mutagenic potency has been reported for several polycyclic aromatic hydrocarbons[3-5]. Wood *et al.*[26] have determined the difference in the mutagenic activities of the (+) and (−) *anti*-BP diol epoxides using a mammalian tissue culture cell line and two bacterial tester strains. The difference in mutagenic activity between the (+) and (−) *anti* diol epoxides in the mammalian cell line was of the same order as we found for the difference in the level of covalent binding of the two isomers to the N^2-guanine site in native DNA. Therefore, the extent to which these activated hydrocarbons react chemically with DNA *in vitro* correlates with their biological activities. These results suggest that, in addition to metabolic factors, chemical and physical interactions with DNA may be important in evaluating the biological potency of carcinogenic and aromatic hydrocarbons.

The regulation of gene expression usually involves protein–nucleic acid interactions[27]. As we have demonstrated highly selective binding between small organic molecules and DNA, the possibility exists that this type of interaction may also have a regulatory role in the normal functioning cell.

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Note added in proof: Of the four isomers, (+) and (−) *anti*- and (+) and (−) *syn*-BP diol epoxides, the (+) *anti*-configuration is the most carcinogenic in newborn mice[28]. Therefore, the extent of non-enzymatic, covalent binding of this enantiomer to double stranded DNA correlates not only with mutagenic activity[26] but also with tumorigenic potency.

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Hydrolysis of protein in vacuoles isolated from higher plant tissue

THE vacuoles of higher plant cells are known to be sites o deposition of salts and metabolites. A role as the lyti compartment has also been proposed[1], and Matile has argue that the vacuole participates in the metabolism of higher plan cells in the same way that lysosomes do in other organism Recently, isolation methods have been developed which allow direct analysis of vacuolar constituents and we have show previously that the vacuoles isolated from the endosperm o young castor bean seedlings contain at least 25% of the cellula protein, 62% of the sucrose and various hydrolytic enzyme (acid protease, carboxypeptidase, phosphodiesterase, RNAase phytase and β-glucosidase) in amounts indicating a primaril (possibly exclusive) vacuolar localisation[2]. In this tissue it i known that when the dry seed imbibes water the outer, water soluble matrix of the protein bodies is dissolved and smal vacuoles containing the protein crystalloid are formed. Thes coalesce early in germination to form the large central vacuole and the remnants of the protein bodies disappear within a fev days as growth proceeds[3]. We have examined vacuoles isolate from the endosperm of 4-d-old seedlings, when it is known that net loss of protein is occurring. Here we compare the protei composition of the vacuoles with that of the origina protein bodies, and show that hydrolysis of th endogenous protein continues in the isolated vacuoles which ar free from other cellular components.

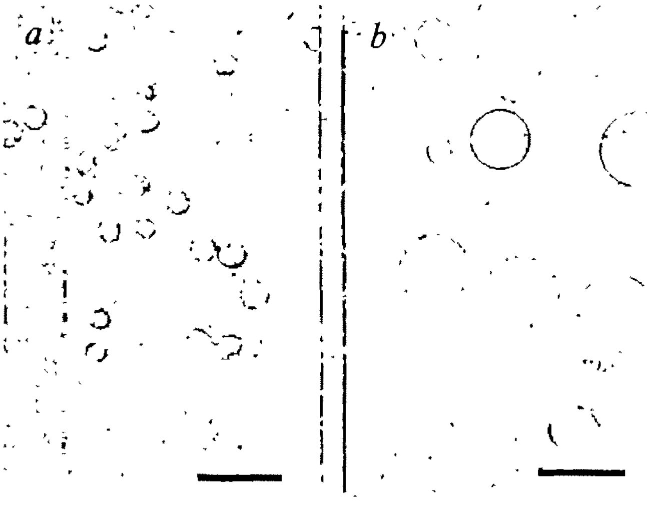

Fig. 1 Photomicrographs of *a*, protein bodies prepared from dry castor beans, and, *b*, vacuoles prepared from endosperm of 4-d-old seedlings and purified by passage into 40% sucrose containing 0.1 mM EDTA[2]. Bars, 50 μm.

Table 1 Protein content of protein bodies and vacuoles

	Protein bodies (mg per 10^6)	Vacuoles (mg per 10^6)
Total protein	0.71	1.92
Water soluble	0.14 (19.7)	0.90 (46.6)
Water insoluble	0.57 (80.3)	1.02 (53.4)

Isolated protein bodies and vacuoles (~1 mg protein in 100 μl) were mixed with 1 ml of water. After removing the insoluble protein by centriguation (10,000*g*, 10 min), the soluble protein was precipitated with 1 ml of 10% trichloroacetic acid, dissolved in NaOH and assayed by the Lowry procedure[7]. The insoluble protein was calculated by subtracting the amount of soluble protein from the amount of total protein determined on NaOH extracts of trichloroacetic acid precipitates of initial samples. Values in parentheses show percentages.

Seeds of castor bean (*Ricinus communis* cv. Hale) were soaked in running water for 1 d and germinated in moist vermiculite at 30 °C. Vacuoles were prepared as described previously[2], using 140 endosperm halves, removed from 4-d-old seedlings. The protein bodies were prepared from 20 dry seeds by the non-aqueous method of Yatsu and Jacks[4]. Figure 1*a*, *b* are photomicrographs of the isolated protein bodies and vacuoles, to the same scale. From analysis of total protein and from haemacytometer counts of appropriate samples, it was determined that the protein content of 10^6 protein bodies was 0.71 mg and that of 10^6 vacuoles 1.92 mg. Thus, at 4 d, each isolated vacuole still contains an amount of protein equivalent to several original protein bodies, as anticipated from the microscopic examination[3].

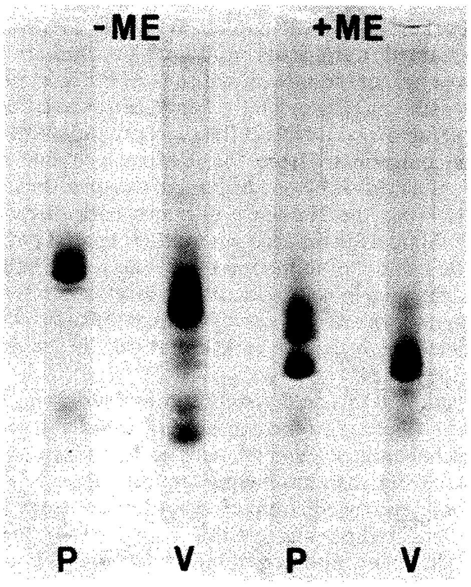

Fig. 2 SDS electrophoretograms of the protein bodies (P) and vacuoles (V) shown in Fig. 1. Samples containing approximately, 70 μg protein were subjected to SDS-polyacrylamide gel electrophoresis in the presence and absence of β-mercaptoethanol (ME) according to Tully and H.B.[5].

As shown in Table 1, only 20% of the total protein in protein bodies is water soluble, compared with 50% in the 4-d vacuoles. This indicates that digestion of the (water-insoluble) crystalloid protein is proceeding. Figure 2 shows typical SDS electrophoretograms of protein bodies and vacuoles. These were carried out as described by Tully and H.B. and approximate molecular weights of components estimated[5]. The whole protein body preparation yielded one major band (mw 60,000) and several minor bands, as previously shown[5]. In the vacuole preparation, the major band had disappeared and new protein bands, with lower mws (40,000, 30,000 and 18,000) were present. In the presence of β-mercaptoethanol, three major bands (mws 41,000, 35,000 and 30,000) were seen after electrophoresis of the whole protein body preparation, whereas two of these (mws 41,000 and 35,000) were not present in the vacuoles. These results show that larger protein components had been converted to smaller polypeptides, as expected from the action of hydrolytic enzymes known to be present in the vacuoles.

The vacuoles contain relatively high amounts of free amino acids (~90 nmol per mg protein). When intact vacuoles, as isolated in 40% sucrose, were incubated at 37 °C, the amount of free amino acid increased with time (Fig. 3*a*), showing that hydrolysis of endogenous protein was sustained in isolation.

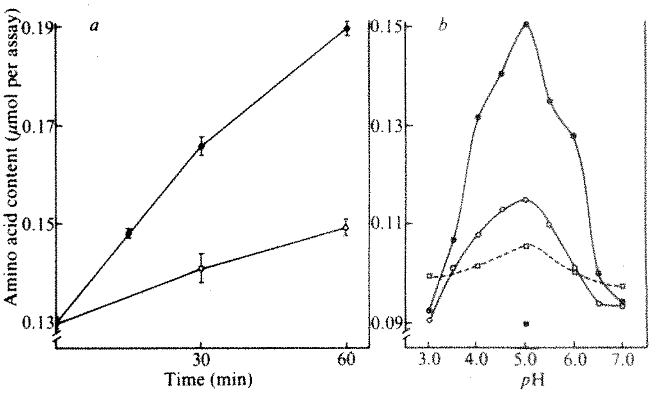

Fig. 3 *a*, Progress of amino acid accumulation is isolated vacuoles. For intact vacuoles (0), 40 μl of 50 mM citrate buffer, *p*H 5.0, in 40% sucrose was added to 10-μl samples of vacuolar preparation containing 156 μg protein. To obtain lysed vacuoles (●), 40 μl citrate buffer, *p*H 5.0, without sucrose was added to similar aliquots of vacuoles. Samples were incubated at 37 °C and the reaction terminated with 50 μl 10% trichloroacetic acid. After centrifugation the supernatant solutions were analysed for free amino acid using the ninhydrin method[6], with glycine as standard. *b*, Aliquots of a vacuolar preparation (105 μg protein) were treated as above to give intact and lysed vacuoles; the *p*H of the citrate buffer was varied as shown. Intact vacuoles were incubated for 30 min (□), and lysed vacuoles for 30 min (○) and 60 min (●) before amino acid analysis. A zero time control (■) is also shown.

Much higher autolytic activities were observed when the sucrose concentration of the medium was reduced by a four-fold increment of citrate buffer. As shown in Fig. 3*b*, the optimum *p*H for autolysis in both intact and lysed vacuoles was 5.0, although the response of the lysed vacuoles showed a much sharper peak.

Thus, the vacuoles show evidence that hydrolysis of endogenous protein has occurred within them before isolation and that this process is sustained in isolation. This seems to be the first direct demonstration of the proteolytic function of these organelles in higher plants.

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Beech bark disease and archaeological crop marks

THE presence of 'crop marks' in aerial photographs is frequently used to detect archaeological features. These marks are produced by differences in plant size or colour which are related to the characteristics of the soil overlying or comprising the man-made feature. Pure stands of many arable crop species are known to show visible responses to soil heterogeneity. In the case of woody perennials we have found only one report describing the detection of archaeological structures by virtue of their influence on the growth of a crop[1]. This was the identification of ancient hut sites in Kenyan tea plantations where excess lime caused failure of the crop. We now report the detection of an ancient field system by virtue of its present-day relationship to patterns of a disorder in beech (*Fagus sylvatica* L.).

An aerial photograph of pure beech stands planted between 1920 and 1948 at Marden Forest, West Sussex, UK, shows some intriguing 'geometrical' patterns of chlorosis. In the area shown in Fig. 1 the trees were 25 yr old when the photograph was taken in 1973. A ground survey of this area revealed a series of scarps up to 1.4 m in height on gently sloping ground. The distribution of these scarps and their structure, as revealed by excavation of two of them, suggests that they are the remains of a field system of prehistoric or Romano-British origin. We have surveyed these scarps or lynchets, and Fig. 2 shows their relationship to the pattern of chlorosis plotted by photogrammetric techniques. Most of the affected trees seem to occur immediately below the scarps.

Our original purpose in examining the photograph had been to detect spatial patterns of beech bark disease which is present at this site and is caused by the scale insect *Cryptococcus fagi* Bär. and the fungus *Nectria coccinea* (Pers. ex Fr.) Fries. Like many plant diseases, beech bark disease has been surveyed using aerial photography[4], the symptom which makes such detection possible being foliar chlorosis[3]. This symptom may, however, be caused by other disorders; in particular, a nutritional deficiency in which beeches growing on thin calcareous soils are unable to absorb iron in adequate quantities[2].

The natural occurrence of nutrient imbalances in soil would not normally follow a spatial pattern of the type displayed by the chlorotic trees. At this site the soil is underlain by chalk and, in its undisturbed state, it probably included a shallow capping of slightly acid material. It is generally considered that in field systems of the type found here, gradual erosion over many years of ploughing along the contour has removed soil from the areas below the field boundaries. Apparently this process, together with disturbance of the underlying chalk, has produced a soil alkaline to the surface so that conditions conducive to nutritional chlorosis have arisen very locally and in a geometrical pattern.

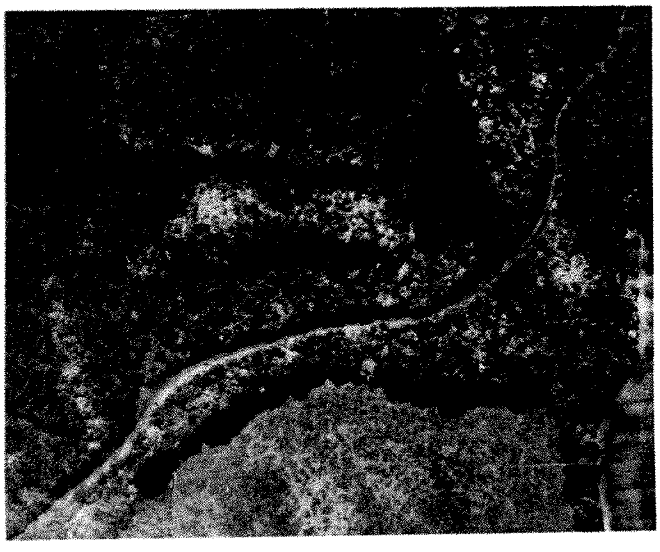

Fig. 1 Aerial photograph of geometrical patterns of foliar chlorosis at Marden Forest. (Crown copyright reserved.)

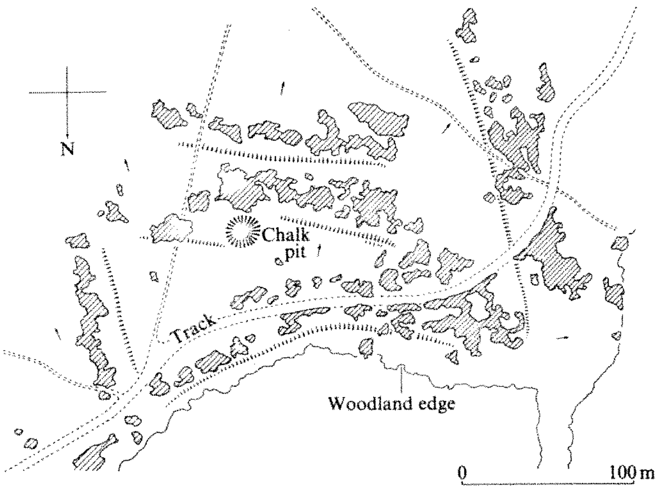

Fig. 2 Map of ancient field system and associated areas of foliar chlorosis. Hachures represent scarps (field boundaries). Cross-hatching represents chlorosis. The arrows indicate the direction of the down-slope.

Aerial detection of beech bark disease in areas where nutritional chlorosis is present would seem to be very difficult, although Parker[5] reported that, in the chalk downland plantations examined during his study of beech bark disease, chlorosis was not due to nutritional causes. Furthermore, there may be interactions between nutritional factors and beech bark disease. Soil factors could affect the susceptibility of the trees to the disease, quite apart from the confusion arising from the production of chlorosis by one or both of the disorders. It would be useful to study the incidence of beech bark disease and of nutritional chlorosis in an area where their spatial patterns are superimposed but can be mapped separately. We believe that the abrupt changes in soil type at the Sussex site offer such an opportunity and we are now surveying in detail the soil variation and the incidence of beech bark disease in the chlorotic and non-chlorotic zones.

The detection of this field system through observing chlorotic tree crowns suggests that the presence of woodland on a site of possible archaeological interest may be less of an obstacle to aerial survey work than has been generally supposed. As far as the detection and prediction of disorders in tree crops are concerned, our findings suggest that knowledge of the ways in which a soil has been modified by man's activities is important to the plant pathologist as well as to those who are responsible for establishing and managing plantations.

We thank Mr H. C. Bowen, of the Royal Commission on Historical Monuments, for discussions.

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reviews

A revaluation of all values

George Steiner

Science in a Free Society. By P. Feyerabend. Pp. 221. (New Left Books: London, 1978.) £7.50.

FORMALLY, this book is a mess. It is a patchwork of polemic, of autobiographical fragments, of re-iterations of or rapid addenda to an earlier book, *Against Method: Outline of an Anarchistic Theory of Knowledge* (New Left Books: London, 1978). In fact, it will make almost no sense to anyone who has not read Paul Feyerabend's main tract or who has not kept its principal arguments and areas of reference clearly in mind. The tone is, once again, truculent, self-serving, caustically dismissive of dissent and, at pivotal moments, rhetorical. A paragraph, chosen almost at random, will communicate the flavour and purpose (one can choose almost at random precisely because the technique of argument is one of circular incantation):

> "Of course, our well conditioned materialistic contemporaries are liable to burst with excitement over events such as the moonshots, the double helix, non-equilibrium thermodynamics. But let us look at the matter from a different point of view, and it becomes a ridiculous exercise in futility. It needed billions of dollars, thousands of well-trained assistants, years of hard work to enable some inarticulate and rather limited contemporaries to perform a few graceless hops in a place nobody in his right mind would think of visiting—a dried out, airless, hot stone. But mystics, using only their minds travelled across the celestial spheres to God himself whom they viewed in all his splendour receiving strength for continuing their lives and enlightenment for themselves and their fellow men. It is only the illiteracy of the general public and of their stern trainers, the intellectuals, and their amazing lack of imagination that makes them reject such comparisons without further ado."

The sleight-of-hand whereby moonshots, work on DNA and the mathematics and/or technology of non-equilibrium thermodynamics are conflated is no less representative of Professor Feyerabend's method than is the bullying lack of punctuation throughout the latter part of the passage.

As readers of *Against Method* will know, the author has two principal and related aims: to demystify the alleged rationality and methodological integrity of the 'exact' sciences and to remove these sciences from the prestigious, politically and socially determinant rôle which they play in current western society. To borrow Nietzsche's tag, Professor Feyerabend is arguing for 'a revaluation of all values':

Paul Feyerabend

Taken from the dust-jacket

> "Bringing a faint smile to the faces of people who have been hurt, disappointed, depressed, who are paralysed by some 'truth' or by the fear of death seems to me an achievement infinitely more important than the most sublime intellectual discovery: Nestroy, George S. Kaufman, Aristophanes, on my scale of values range far above Kant, Einstein and their anaemic imitators."

Feyerabend's position might be defined as that of a 'therapeutic anarchist' (it is very close to that of an educational iconoclast such as Ilich, who figures in the text if not in the index). The enemy, among others, is Popper: Sir Karl is "a mere propagandist" whose teachings and following constitute "a tiny outhouse" (elegance of expression is not among Professor Feyerabend's weaknesses). But the fundamental enemy—and here Feyerabend's polemic is precisely on target—is "the rise of intellectualism in Ancient Greece", and the unfolding of this intellectualism in the methodologies of Descartes and Kant and in the scientific world-image of Newton and his modern inheritors. It is this 'intellectualism', this belief ('illusory, naïve, finally ludicrous, says Feyerabend) in 'objective truths' and in the disinterested passions of abstract, mathematically-grounded theorising, which have brought the west to its current state of confused inhumanity. Trapped in our naïve, wasteful 'scientism', we no longer even have access to the pluralistic possibilities inherent in the 'animism', in the 'mysticism', in the intuitive, mythologically articulate mentalities and wealth of humane insight in cultures, in modes of apprehension which we have ostracised as 'primitive' or 'irrational' (as if the actual proceedings which led to the Copernican revolution or to Einsteinian relativity were not, in their own way, 'primitive' and 'irrational').

The text is most rewarding where it tells of the personal and pedagogic road which led Paul Feyerabend to his current stance. Kierkegaard's anti-rationalism was an incitement. Dada and the expressionist send-ups immediately after World War I were important (Feyerabend is absolutely right when he notes that Dadaists went deeper than anyone else in identifying the creative and 'deconstructive' "miracle of language"). There were Austrian Marxism and the Vienna School, with its ultimately unfulfilled promise of a rigorous yet also accurately descriptive logic of the sciences. And, above all, perhaps, there was California, with its plethora of informalities, instant utopias, 'Orientalisms', 'counter-cultures' and liberating sunshine.

If Feyerabend often knocks down straw-men—who now refuses to argue the status of the exact sciences, what serious mind is not worried about the technocratic enormities in our environment and politics?—he also scores shrewd hits. He is acute on the innocent 'scientism' of the Vienna Circle; his critique of positivistic paradigms amends and deepens Kuhn. He is robustly anti-parochial, following Protagoras in the conviction that a pluralistic view of values and ideals is the only way of stepping beyond one's village towards the human community at large. Thus, Professor Feyerabend's plea for a new catholicity of understanding is often poignant and persuasive. It is also, now and again, great fun. That a more patient sensibility, that a style of inquiry less contemptuous of disagreement, would give to *Against Method* a more effective place in the present climate of discourse, are considerations which, one fears, Paul Feyerabend would regard as time-serving, silly or both. The dust-jacket photo of the faintly sneering author seems to declare as much. This is a pity, for the issues raised are of the first importance. □

George Steiner is Professor of English and Comparative Literature at the University of Geneva, Switzerland.

Correlation of morphology with chemical composition

Electron Probe Microanalysis in Biology. Edited by D. A. Erasmus. Pp. 248. (Chapman and Hall· London, 1978) £15.

OFFERING the capability of chemical analysis *in situ* at a subcellular level, electron-probe X-ray microanalysis is an exceptionally effective means for the correlation of morphology and chemical composition Although the importance of the technique in biology is now well established, there are few texts giving the newcomer all-around guidance in its biological use Indeed, aside from the volume under review, only the 1977 book by John Chandler comes to mind

The editor's introduction is followed by a capable exposition by J C Russ of physical fundamentals and limits of performance The next two chapters are by J. A Chandler ("The application of X-ray microanalysis in TEM to the study of ultrathin biological specimens—a review") and by A. J Morgan, T W Davies and D. A Erasmus ("Specimen preparation"). Although there is considerable overlap between these sections, Chandler's chapter features a very extensive coverage of the literature on biological applications, whereas Morgan *et al.*, provide a much fuller comparison of a variety of preparative procedures The chapter by T. C. Appleton ("The contribution of cryo-ultramicrotomy to X-ray microanalysis in biology"), is mainly a good up-to-date presentation of the difficult procedure of preparing ultrathin cryosections of quench-frozen material In the following chapter ("The application of X-ray microanalysis to histochemistry"), I. D. Bowen and T. A. Ryder give a critical review of precipitation techniques with particular attention to enzyme localisation The final section, "X-ray analysis applied to the study of renal tubular fluid samples" by H O Garland, J. A. Brown and I. W Henderson, describes the special techniques used for the quantitative analysis of tiny samples of fluid obtained by micropuncture and documents the effectiveness of the electron-probe method in this field

Two limitations of the text should be noted. Firstly, as the editor states, "The field is . enormous and in order to follow certain aspects in depth, the contents of this book have been restricted essentially to the analysis of specimens in the transmission electron microscope." Nevertheless, although the emphasis on transmission microscopy is plainly evident, much of the contents, especially with regard to specimen preparation, applies equally well to studies based on scanning electron microscopy, and indeed the published X-ray analyses of micropuncture specimens have generally been done with scanning-type instruments

Secondly, as an over-view, the text is very limited in the area of studies associated with cryotechniques, the quantitative analysis of diffusible elements, and the attempt to control radiation damage by means of low temperatures Although Appleton's chapter gives an excellent summary of procedures for preparing cryosections, major results from several laboratories on the quantitative analysis of diffusible elements in cryosections have been published too recently for inclusion

However, the analysis of diffusible elements in cryosections is a field still in its infancy, and in any case requires specialised facilities. For the biological electron microscopist whose EM column is fitted with X-ray microanalytical facilities, the book gives a good idea of what can be done relatively readily, and how to begin doing it

T. A. Hall

T A Hall is Assistant Director of Research in the Department of Zoology, University of Cambridge, UK

Erratum

The review of *A Review of Amino*
id Transport Processes in Animal
and Tissues (*Nature*, 4 January;
1979) was by C. W I. Owens,
ecturer in Clinical Medicine,
stated.

Selective controlled fusions of membranes

Cell Surface Reviews. Vol. 5· Membrane Fusion. Edited by G. Poste and G. L. Nicholson Pp. 862. (North-Holland New York and Oxford, 1978.) $120

SELECTIVE, controlled fusions of membranes have long been recognised as major features of cellular and subcellular interactions in living organisms but only in recent years has any concerted effort been made to establish their significance and to identify the molecular events involved. A comprehensive treatment both of the phenomena and of attempts to account for them at the molecular level is thus both opportune and welcome.

Poste and Nicolson have made excellent choices of topics and authors to provide this comprehensive coverage, and the individual authors (with one or two exceptions) have poured all they had into it The result is a rather large book that takes a long time to read, but the articles and their extensive reference lists (with titles) provide a reservoir of information which those directly involved in fusion studies will explore with relish and at which those with more limited interests will nibble with profit.

Most of the book is inevitably taken up with descriptions of the circumstances and conditions in which membrane fusion takes place both *in vivo* and *in vitro* Fusion events involved in fertilisation in invertebrates (chapter 1) and in vertebrates (chapter 2) are discussed in largely phenomenological terms. Somatic and sexual fusions in myxomycetes and in fungi (chapter 5) are more amenable to studies of control factors, as also are myoblast fusion (chapter 3) and macrophage fusion (chapter 4) The latter can be studied in culture conditions as well as *in vivo.* Plant cell protoplasts also provide a useful system for the study of the conditions required for cell fusion (chapter 8) now that effective means of removing cell walls have been devised. Fusion events which transfer both vesicle-enclosed material and membrane itself through the intracellular system during endocytosis and/or exocytosis are also extensively covered (chapters 9–12).

Detailed descriptions of chemically induced (chapter 6), virus-induced (chapter 7) and Ca^{2+}-induced (chapter 8) fusions *in vitro*, provide a basis for speculations concerning factors which may contribute to fusion processes *in vivo* Throughout the book there are repeated references to possible influences of the nature and distribution of lipid components, the nature and distribution of protein and glycoprotein components and levels of Ca^{2+} ions on a variety of fusion processes, and there is a final chapter on problems in the physical interpretation of membrane interactions and fusion.

Thus this volume provides an authoritative, detailed and up-to-date assessment of studies relating to membrane fusion which will contribute substantially both to our understanding of and experimental approach to the problem

J. B. Finean

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Transform techniques in chemistry

Transform Techniques in Chemistry Edited by P. R. Griffiths. Pp. 385. (Plenum: New York and London, 1978) $47.40.

MANY spectroscopists, particularly those using nuclear magnetic resonance, have had their field revolutionised in the past few years by the introduction of Fourier transform techniques but have been forced to pick up the new folklore in a haphazard manner for lack of a suitable general treatment of transform methods written from the point of view of the chemist. This excellent new book goes a long way towards satisfying this need It provides a convincing demonstration that the concept of Fourier transformation is common to a whole range of physical techniques, ranging from the well-known NMR spectroscopy and infra-red interferometry to the more recent Fourier transform ion cyclotron resonance Although Fourier transformation naturally dominates the discussion, it is shown that related techniques such as the Hadamard and Walsh transforms can also be useful to the spectroscopist in suitable circumstances.

This book is a collection of chapters by different authors, and can be divided into the mathematical and data handling topics (chapters 2–4, 7, and 11–13) and applications to specific types of spectroscopy or chemistry (chapters 1, 5, 6, 8–10 and 14). This method of compiling a book has the obvious strength of allowing the important sections to be written by the appropriate expert; in the present case there is an excellent description of Fourier transform ion cyclotron resonance by Comisarow. On the other hand, multiple authorship can mean undesirable repetition. Worse still, a given topic may be treated superficially several times over, no single author wishing to devote enough space for a definitive discussion. For example, the practice of 'zero-filling' interferograms raises some fairly fundamental questions that are seldom answered to the satisfaction of the average spectroscopist, and one would have expected the present volume to tackle this hot potato once and for all. Instead the subject is treated separately in chapters 4, 8 and 11, in each case rather uncritically.

This is an extremely useful book for locating those elusive but vital pieces of information the research worker always seems to be looking for; at the same time the general concepts are very well treated. Most readers will be surprised at the number of different areas where transform techniques are used and how related problems turn up in different forms in the different fields This broadening of our understanding could well lead to new experiments inspired by the methods used by our colleagues. Everyone will find something new in this book Even the jargon of our fellow spectroscopists can come as a surprise; the reviewer particularly noticed "dichotomizer", "cross-correlogram", "decimation" and "transgeneration".

In spite of the use of multiple authors, the material presented is remarkably up-to-date, and there seem to be no glaring omissions It would have been nice, however, to have seen some mention of Fourier transform methods of NMR imaging (zeugmatography) and the developing field of two-dimensional Fourier transformation.

Ray Freeman

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Soil profile recording

Soil Sampling and Soil Description By J. M. Hodgson. Pp 241 (Clarendon Oxford University Press Oxford, 1978.) £10.

FROM the outset it is essential to realise that this little book is not intended to be a field handbook Rather it is a review of 'how and why' specific profile features should be recorded together with a summary of the descriptive methods adopted by various national and international organisations

In the opening chapter the author considers the reasons for studying the soil in the field and the need to describe accurately profile features Chapters 2 and 3 provide a concise review of methods of profile site selection, description and preparation.

The 'how and why' of soil description is the central theme of chapter 4, which provides a comprehensive list of the soil features the field scientist is likely to encounter Unfortunately the approach adopted in this chapter is by no means uniform Each feature is dealt with in turn, in some instances the reasons for selecting a particular feature are discussed in detail, whereas in others the importance is assumed to be obvious Similarly when dealing with possible means of measuring or classifying a particular feature the methods are sometimes considered in detail, as for example in the case of structure or as in the case of roots only perfunctorily.

This long account is followed by two succinct chapters covering techniques of data recording and sample collection Of particular value is the section on recording of data in a form amenable to computer storage. (In this section figures 5 5 and 5.8 are transposed) The final chapter is in the form of a summary of the profile features used in soil description by some 28 different organisations The reviews are necessarily brief and details are only given where the procedure or feature used differs from those in the USDA 1951 Handbook. This chapter is valuable if only because it brings together for the first time the approaches adopted by a variety of agencies.

Overall one is left with the question of what is the purpose of this book? Had the chapters on the 'how and why' of soil description considered the question of 'why' in greater depth then this would indeed have been a useful text

M. J. Alexander

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Quantum chemistry

Quantum Chemistry. (Reference Edition.) By J. P. Lowe. Pp. 599. (Academic: New York, San Francisco and London, 1978.) $49.50; £32.15.

QUANTUM CHEMISTRY signifies different things to different minds. Some forget the 'chemistry' and provide for chemists simplified, or simply curtailed, versions of books on quantum mechanics. Some concentrate on molecular properties with and without spectroscopy; others concentrate on molecular structure. The last are increasingly based on computer techniques—as they should be—for they have provided the most important quantitative advances in recent years. In them lies the hope of simulating more precisely the processes that occur when molecules undergo the dramatic changes that result in reaction, and in them lies one of the very few tests we possess of the qualitative ideas that underlie our understanding of the equilibrium geometries of molecules as well as their reaction characteristics.

Dr Lowe has written a book that covers parts of this well trodden ground. It is aimed principally at readers whose interest is in molecular structure, and to say what is not in it may be as helpful as saying what is in it: thus it is not a spectroscopy book, nor is it a molecular properties book. It does, however, deal with basic quantum mechanics (but reserves operator properties until well on into the text), atomic structure, and then, for over half the book, molecular structure. The level of treatment of molecules is slightly beyond what undergraduates normally meet, and covers basic LCAO–MO theory, Hückel theory, and SCF–MO theory. The final chapter, which I suspect the author is proud of most, is on 'qualitative molecular orbital theory', which turns out to be the Walsh-type argument for structure and Woodward–Hoffmann rules for reactivity.

Although the basic material is treated in other books with similar titles, there are aspects of the treatment that make the book attractive. The author clearly has an eye for the interesting analogy and felicitous example, and readers will enjoy the sensitivity of his exposition. There are places where he apparently has not troubled to think up an explanation (for example, the Hund rules), but on the whole readers will find themselves in the presence of an imaginative and reliable interpreter.

At various points I thought the author's judgement faltered. For instance, I think very little purpose is served by working through in detail the old-fashioned way of solving the harmonic oscillator Schrödinger equation; and even less is served by sketching the hydrogen atom solution before talking about angular momentum, leaving angular momentum itself largely unexplained, and being unhelpful about spin. Hückel theory is old hat, but it was nice to see modern judgements (and comparison with experiments, as in hyperfine coupling constants) brought into the discussion. Various nice examples of perturbation theory are presented, and group theory is presented in a suitably pragmatic way.

In summary, Dr Lowe has written a nice book, which covers old ground in a pedagogically attractive and largely contemporary way. Readers will find it an agreeable, accessible commentary on modern structural quantum chemistry.

P. W. Atkins

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Insect behaviour

Insect Behavior. By R. W. Matthews and J. R. Matthews. Pp. 507. (John Wiley: Chichester, UK; and New York, 1978.) £15.85.

IT is rare to find a major gap in the present plethora of scientific books but a treatise on insect behaviour has been badly needed for years. There is surely more published work on this subject than on the behaviour of any other animal group. A great part is descriptive and no small part anecdotal but insect behaviour has also been the subject of innumerable analytical studies, the special strength of which has been their leaning toward physiology rather than psychology.

This book makes a bold and within its purpose successful attempt to fill the gap. Faced with the superabundance of information and the infinite diversity of insect behaviour the authors had to choose between breadth and depth, and chose breadth. Comprehensiveness probably was in fact the most pressing requirement for a first book in the field, one that would serve as a detailed 'guidebook' for entomologists and behaviourists generally.

Inevitably it will be said that in trying to cover everything the authors succeed in saying little about anything. Again and again the reader is tantalised by the opening of an interesting topic only to find it closed again for another. However, while holding to their comprehensive purpose the authors have gone some way to meeting this criticism by frequently interpolating fuller 'case-studies', and by ending every small subsection with citation of up-to-date reviews and books on that specific theme, an invaluable feature. It is difficult to think of any topic that does not get some introductory treatment in the book: slave-making and Zeitgebers, cannibalism and coyness, heritability, flicker-fusion, the r–K spectrum, Solanum alkaloids, stimulus filtering, bioluminescence, parental investment . . . it is all there. The large Subject Index unfortunately does not adequately underpin the guidebook function, the bulk of it being insect names. Let us hope the authors will provide a fuller one in future editions.

The authors describe the general approach of their book as "ethological", meaning a primary concern with descriptions, functions, behavioural ecology and evolution. After an introductory chapter including a warning against anthromorphism and teleology, one chapter of 40 pages deals sketchily with nervous and hormonal mechanisms. The rest of the book is devoted to the gamut of behavioural activities under two main heads, "maintenance activities" of individuals, and "communicatory activities" between individuals of the same or different species. Maintenance is covered in chapters on "Spatial Adjustment" (locomotion, orientation, dispersal and migration) and "Feeding Behavior" (plant relations, food location, social feeding, food recognition and feeding regulation).

Communicatory activities take up the remaining 300 pages out of the total of 500, an emphasis reflecting the enormously increased recent academic and practical interest in these matters. Chemical, visual and mechanical (including acoustic) communication each have a chapter followed by three more strictly focussed on activity types, defensive and reproductive behaviour, brood care and social life. The arrangement is novel and effective. Illustrations are profuse, one on almost every other page, and include many photographs which have not all reproduced well. The reviewer has found one or two erroneous passages and it would be astonishing if there were not more in such an ambitious undertaking. There is naturally a certain transatlantic bias: it is strange, for example, not to find F Huber's work in an account of acoustic communication.

The book remains a monumental guidebook which insect and other behaviourists will want to have access to for years to come.

J. S. Kennedy

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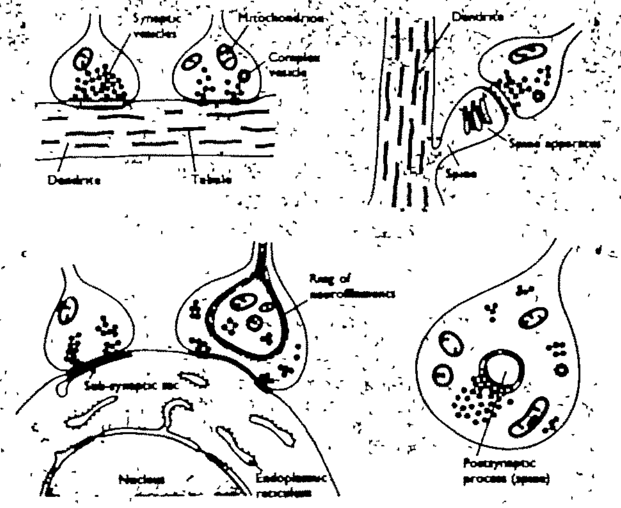

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Fish-finding echosounders. Marconi Marine. The Chromascope K range of echosounders from Marconi Marine is designed to display received echoes from fish shoals and the seabed in colour on a television-type screen. Received echoes, fed from a storage memory circuit, are displayed in colours and tints which directly relate to the intensities of the signals received from individual objects. The result is a coloured mosaic of signals which integrate to form a chart-like presentation which shows fish shoals and the seabed in a manner which is easy to interpret. The integrated chart is constantly updated from the memory store.
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UV-vis spectrophotometer. Perkin-Elmer. In the model 552, UV-vis spectrometer from Perkin-Elmer, operation is both fast and simple. Concentration is automatically set by the keyboard and microcomputer. When a wavelength is keyed in, the monochromator rapidly sets itself at the press of a button. Both ordinate and abscissa data are clearly shown on large digital displays, and operating parameters such as mode, scan speed, format and response are conveniently displayed. The 552's automatic baseline compensation mode ensures baseline flatness of ± 0.001 A. An optional keyboard expander provides enhanced correction capacity and the ability to compensate over preselected wavelength intervals.
Circle No. 54 on Reader Enquiry Card.

Digital electronic thermometer. British Rototherm. British Rototherm have now introduced a compact electronic digital thermometer which combines accuracy, convenience and rugged reliability at a keen price. Designated the electrotherm, it resembles a conventional pocket calculator and is supplied complete with an easy-to-clean probe which can be stored in a built-in holder when not in use.
Circle No. 55 on Reader Enquiry Card.

Dewpoint meter. Shaw. The new Shaw automatic dewpoint meter uses room air moisture as a standard for rapid, automatic calibration to ensure that the sensor is fully responsive, without drift. The dewpoint meter requires only 4 to 5 minutes to reach equilibrium moisture readings even at very dry levels. The sensor is internally mounted within a desiccant chamber providing automatic dry-down between tests. This feature ensures that the meter is always ready to check dryness even down to 1 or 2 p.p.m. of water vapour. A direct-reading analog meter displays dewpoint, on a linear scale. Accuracy of reading is within ±1 p.p.m. or ±3 °C dewpoint, whichever is greater over the range from −80 to −25 °C dewpoint.
Circle No. 56 on Reader Enquiry Card.

Laboratory furniture. Sintacel. Metriscope from Sintacel is a comprehensive range of equipment capable of fulfilling the servicing and furnishing requirements of educational and laboratory accommodation. The range provides non-fixed furniture with overhead service booms and drainage equipment. All furniture is either movable or mobile and the working surfaces, with their flexible service/drainage connections, can be quickly re-arranged. The overhead service booms may provide such services as gas/electricity/water/vacuum/lighting. Drainage is normally dealt with by means of a regularly spaced grid of floor outlet points, but if so required, wall or overhead drainage may be provided.
Circle No. 57 on Reader Enquiry Card.

Laser systems. Coherent. Three new laser systems have been introduced by Coherent. The first of these, the CR-599-04 delay line dye laser, is a self-contained dye laser designed for synchronous pumping, and has an overall laser length of only 0.6 m. It is pumped by an actively mode locked ion laser, enabling optimal tuning range, stability and average output. The second system, the Series 467-SE, is a mode locking system consisting of an ultrastable RF oscillator/amplifier whose frequency is synthesised from a temperature-stable crystal oscillator reference, coupled to an acousto-optic modulator which mounts to any CR series ion laser. The third system, the CR-599-03E scanning dye laser, has an Etalon Servo lock to minimise mode hops, and has an operating range of 415–850 nm.
Circle No. 58 on Reader Enquiry Card.

Heating/stirring reaction module. Pierce. Pierce announces the introduction of their Reacti-therm heating/stirring module. The new dry block module is designed for use in the preparation of derivatives for GC and LC, for sample concentration, residue isolation and protein hydrolysis. A key feature of the unit is a continuously variable magnetic stirrer which is especially useful for dissolving sticky, hard-to-dissolve residues and for accelerating derivatisation reactions. The unit also has an ambient to 150 °C temperature range with ±0.5 °C control, a temperature stabilisation indicating light and a choice of interchangeable vial-holding aluminium blocks.
Circle No. 59 on Reader Enquiry Card.

Refrigerated constant temperature bath. Haake. The new heavy duty refrigerated constant temperature bath and circulator, model F3-K, from Haake, permits low temperature operation down to −30 °C. The unit has been specifically designed to control the temperature of laboratory objects where large amounts of heat must be removed. The refrigeration system offers a cooling capacity of 320 W at +20 °C (260 W at 0 °C). The integral temperature control unit provides continuous optimum control. Additional features of model F3-K include digital temperature setting, platinum resistance sensor (Pt100) and two safety devices for high temperature and minimum liquid level in the instrument's bath container.
Circle No. 60 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

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GRAVITATIONAL-WAVE DETECTION AND EXPERIMENTAL GRAVITY—CALTECH

The California Institute of Technology is creating a new research group in gravitational wave detection and experimental gravity. Various investigations related to gravitational radiation are being planned, using laser interferometry, cryogenic electronics, or other techniques; and correlated experiments over a 7,600 kilometre baseline between California and Scotland may be performed. The group will be in a good position for close collaboration with Caltech's gravitation theory group, and also for pursuing gravity experiments in space in association with Caltech's Jet Propulsion Laboratory.

Inquiries are invited from outstanding experimental physicists, engineers, or technicians who might wish to join this new group. It is anticipated that the following types of appointments may be made in 1979 and 1980: research staff at levels equivalent to assistant, associate, or full professor but without teaching duties; assistant professorships; postdoctoral fellowships; and technicians. Inquiries from women and minorities are particularly encouraged.

Inquiries — including curriculum vitae, lists of publications, and letters of recommendations sent independently —should be sent to Professor Ronald W. P. Drever, 130-33 Caltech, Pasadena, California 91125, or Department of Natural Philosophy, Glasgow University Glasgow G12 8QQ, Scotland.
9411(A)

UNIVERSITY OF GLASGOW LECTURESHIP IN PHARMACOLOGY

Applications are invited from medically and/or scientifically qualified pharmacologists for a Lectureship in Pharmacology. The salary will be within the range £3,883 to £5,604 of the Lecturers' scale £3,883 to £7,754 (interim), with placement according to qualifications and experience.

Further particulars may be obtained from the Secretary of the University Court (Room 18), University of Glasgow, Glasgow C12 8QQ, with whom applications (eight copies), giving the names and addresses of three referees, should be lodged on or before February 16, 1979.

In reply please quote Ref. No. 4263/1M. 9414(A)

ST PATRICK'S COLLEGE
Maynooth
(Recognised College of the National University of Ireland)
LECTURER/JUNIOR LECTURER IN MATHEMATICAL PHYSICS

Prior to application, further details may be obtained from the Secretary, Academic Council, St Patrick's College, Maynooth, Co. Kildare, Ireland.

Salary Scales: Lecturer: £6,035 to £7,157; Junior Lecturer: £4,493 to £5,237. 9396(A)

Anglian Water Authority
ASSISTANT TEAM LEADER
(Mathematics)
Post Ref: H6R003 Grade 6
£5,133–£5,493 p.a.

Applications are invited for the above post currently based at the Great Ouse River Divisional offices, Cambridge. The successful applicant should preferably be a graduate in mathematics with experience in the water quality modelling of estuaries, coastal waters and reservoirs, and the organisation and collection of associated data. In addition to his individual projects, the successful applicant will also be expected to assist other team members in all aspects of their varied chemical and biological investigations, including computer programming, data processing and statistics.
The post carried a Regular User Car Allowance and N.J.C. conditions of service, including a contributary pension scheme, apply.

Please write, or telephone 0223 61561 ext. 259 for an application form (returnable by Friday 23rd February, 1979) to Mr. M. J. Pearson, Regional Research Team Leader:—

GREAT OUSE RIVER DIVISION,
Great Ouse House, Clarendon Road,
Cambridge, CB2 2BL 9413(A)

APPOINTMENTS VACANT

THE ROYAL VETERINARY COLLEGE
University of London
Division of Preclinical Studies
DEPARTMENT OF ANATOMY
LECTURER IN ANATOMY

Applications are invited for the above post which is available from October 1, 1979.

The Department has responsibility for teaching the topographical and systematic anatomy of the domesticated animals and provides a more general course in Histology and Embryology.

The person appointed will be expected eventually to undertake integrated teaching of these components but will have immediate responsibilities for the teaching of gross anatomy of large animals. The appointee will be encouraged to engage in research.

The post is based at Camden Town.

Salary Scale (under review): £4,333 to £8,204 including London Allowance. Initial salary determined by experience and qualifications. Superannuation under the Universities' scheme.

Removal expenses up to £800 may be reimbursed in approved circumstances.

Application form and further details from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. Telephone 01-387 2898.

CLOSING DATE FOR APPLICATIONS: March 5, 1979. 9419(A)

IMPERIAL COLLEGE
(University of London)
DEPARTMENT OF ZOOLOGY AND APPLIED ENTOMOLOGY

Applications are invited for two temporary Lectureships in the general field of Biomathematics, Applied Statistics and Systems Analysis. The persons appointed will be expected to contribute to the undergraduate and postgraduate teaching and to participate in the application of mathematical and statistical techniques in biological research. One of the posts will be related to the work of the Environmental Management Unit, which is concerned with the application of systems analysis, decision analysis and mathematical modelling to problems of Environmental Management A first degree in a mathematical, physical or biological subject is required, with relevant postgraduate experience.

Appointments will be made for two years at a point in the lecture scale (£3,883 to £7,754 plus London Weighting if appropriate: subject to review).

Applications in duplicate, with curriculum vitae and the names of two referees, should be addressed to the Head of Department, Department of Zoology, Imperial College, London SW7 2AZ, from whom further particulars may be obtained. Closing date for applications: March 1, 1979.
9417(A)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following:

Research School of Physical Sciences
SENIOR FELLOW/FELLOW/SENIOR RESEARCH FELLOW/RESEARCH FELLOW/POSTDOCTORAL FELLOW
DEPARTMENT OF SOLID STATE PHYSICS

The department, formed in 1971, currently has an academic staff of eight headed by Professor W. A. Runciman, and carries out research centred at present on spectroscopic studies of 3d, 4f and 5f transition metal ions in solids at low temperatures. Much of the completed experimental work has been carried out on 5f and 3d ions as well as colour centres in substituted oxides, synthetic and natural minerals. Theoretical studies in the Department have been aimed at providing detailed interpretations of spectroscopic parameters in terms of the underlying electronic processes. Several of the current experimental projects were designed explicitly to provide tests of these interpretations.

Applicants will be considered with previous research experience either in experimental spectroscopy (optical, Mossbauer or EPR) or in measurements of the structural or magnetic properties of metallic materials.

CLOSING DATE: **February 28, 1979.**

SALARIES:

Salary on appointment will be in accordance with qualifications and experience within the ranges: Senior Fellow $A23,737 to $A27,660 p.a.; Fellow $A17,694 to $A23,704 p.a.; Senior Research Fellow $A21,201 to $A25,289 p.a.; Research Fellow $A15,179 to $A19,813 p.a.; Postdoctoral Fellow $A15,179 to $A19,813 p.a. Current exchange rates $A1= $US1.14: £0.58.

TENURE:

Appointment as Senior Fellow/Fellow is five years in the first instance with the possibility, after review, of reappointment to retiring age 65 years; as Senior Research Fellow/Research Fellow normally for five years in the first instance with the possibility of extension to five years; as Postdoctoral Fellow for a maximum of two years.

OTHER CONDITIONS:

Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain further particulars from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. 9400(A)

UNIVERSITY OF WOLLONGONG
Australia

LECTURER IN CHEMISTRY
(Limited Term)

Applications are invited for a ecturership in the fields of analytical and organic chemistry for duties associated with undergraduate and postgraduate courses The appointment will be made for a period not exceeding four years. Applicants should hold . Ph.D. degree or its equivalent and preferably have had some postdoctoral research experience in organic mass spectroscopy and/or trace nalysis The successful candidate will be expected to establish a research programme actively utilising the department's mass spectroscopy facility

Further information may be obtained from Professor B Halpern, Chairman, Department of Chemistry, in the University

Closing date **March 31, 1979.**

SENIOR LECTURER/ LECTURER IN COMPUTING SCIENCE (Tenurable)

Applicants should have a Ph D in Computing Science or equivalent qualifications together with demonstrable research ability and experience Preference may be given to applicants with experience in such areas as operating systems, computer aided learning or software tools

The Computing Science Laboratory ontains 16 terminals timesharing an nterdata 7/32 computer under UNIX The main computer is a UNIVAC 106 Both systems are available for teaching and research A Microomputer laboratory is under development

Further information may be obtained from Professor Juris Reinfelds, Chairman, Department of Computing Science, in the University

Closing date **March 1, 1979.**

Salary ranges Lecturer $A15,786 to A20,737, Senior Lecturer $A21,180 to A24,687, commencing salary according to qualifications and experience Fares to Australia (for appointees and dependants) and a relocation allowance are paid For a limited term appointment a contribution towards repatriation fares on conclusion of appointment may be made

Further particulars and application rocedure obtainable from the Association of Commonwealth Universities Appts.), 36 Gordon Square, London VC1H 0PF 9420(A)

UNIVERSITY OF SOUTHAMPTON

POSTDOCTORAL RESEARCH FELLOW

DEPARTMENT OF BIOLOGY

Applications are invited from biologists with interests in Taxonomy to work with Dr F. A Bisby and Dr J White for three years commencing March/May 1979 The S R C unded project will involve building a data-base with morphological and chemical legume data and experimenting with the provision of novel taxonomic services for evaluation by a users' panel The successful applicant will have an interest in the use of computer-based techniques in taxonomic problems

Starting salary from £3,883 to 4,382 plus U S S benefits

Further particulars may be obtained from Mrs E C P Sears, Staffing Section, The University, Highfield, outhampton SO9 5NH to whom applications with curriculum vitae and names of two referees should be sent as soon as possible, and not later than March 2, 1979 Please quote reference number 1707/R/Na. 9401(A)

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unilever research

Scientists – Animal Feedstuffs

Due to reorganisation we have the following four vacancies in our Raw Materials and Animal Production Section:

Monogastric Nutritionist (Ref: CH-233M)
The job holder will plan and direct the work programme of a small research team carrying out studies on monogastric metabolism/nutrition with the objective of further understanding the relationships between diet and animal performance. This will include the development of experimental techniques for studying protein metabolism in monogastrics and computer simulation in animal growth.

Candidates should have an honours degree in biochemistry/nutrition with research experience to Ph.D. or similar level in pig or poultry nutrition. Experience in computing would be advantageous.

Ruminant Nutritionist (Ref: CH-234M)
The job holder will plan and direct the work programme of a small research team carrying out studies on ruminant metabolism with the objective of improving utilization of raw materials in feedstuffs.

Candidates should have an honours degree in agricultural science/nutrition with research experience to Ph.D. or similar level in animal nutrition with particular emphasis on ruminants.

Raw Materials Biochemist – Animal Feedstuffs
(Ref: CH-235M)
The general area of work is raw materials in support of Unilever's animal feedstuffs business. The work involves evaluation of raw materials and development of methods for improving their nutritive value for use in animal feeds, mainly for ruminants.

Candidates should have an honours degree in biochemistry with research experience to Ph.D. or similar level in plant science, preferably with particular emphasis on protein, carbohydrate or lipid structure and metabolism. An appreciation of farm animal nutrition and experimentation would be useful but not essential.

Nutritional Biochemist – Animal Feedstuffs
(Ref: CH-493A)
The general area of work is the exploitation of fats in animal feedstuffs. The research programme covers two main aspects:

(a) The evaluation of fats as energy sources for pigs, poultry and ruminants.
(b) The effects of fats in diets on primary and secondary production characteristics (e.g. carcass composition) of farm species, and their interaction with other dietary components.

Candidates should have a good honours degree or M.Sc. in biochemistry with chemistry and preferably with some experience or knowledge of lipid biochemistry/metabolism.

A good starting salary dependent on qualifications and experience and other Unilever benefits of employment will be offered to the successful candidate.

Please write for an application form and quoting reference number to:

Miss J. Kenny,
Recruitment and Career
Development Manager,
Unilever Research,
Colworth Laboratory,
Unilever UK Central Resources Ltd.,
Colworth House, Sharnbrook,
Bedford MK44 1LQ.

9402(A)

Universiteit van Amsterdam

Applications are invited for a teaching and research post

lecturer (m/f)

(wetenschappelijk medewerk(st)er)

in the Department of Structural Geology.

Duties will include field and laboratory courses in theory and practice of analytic structural geology, and research.
Applicants should have experience in small scale tectonics. Experience of work on the relationship deformation/metamorphism will be an asset.
Salary in accordance with background and experience.

Applications, with curriculum vitae, precise research interests and the names of at least two referees, should be sent to:
Prof. Dr. C. G. Egeler, Geologisch Instituut,
Nieuwe Prinsengracht 130,
1018 VZ Amsterdam, the Netherlands
(quoting number 3013).

9403(A)

CENTRAL PUBLIC HEALTH LABORATORY

National Collection of Type Cultures (N.C.T.C.)

SYSTEMS DESIGNER/ PROGRAMMER

A systems designer/programmer, preferably a science graduate, is required for the operation of a bacterial identification service Bacteria from clinical sources are identified by probablistic analysis of test results by reference to computer-stored data bases Systems use Optical Mark Reader forms, Data Recognition (Dataterm 3) Machine, Extel punch tape terminal connected to the LUCS 6500 time sharing system, current software in FORTRAN IV There is considerable scope for improving the responsivness of the system and the efficient storage and retrieval of data, and to revise the methodology or explore alternative methods Sympathetic consideration would be given for specialised training, if required

Grading dependant upon basic qualifications in appropriate subjects and on experience

Salary scales Scientific Probationary scale £2,991 to £4,275 plus London Weighting (£354), Post Probationary scale £3,798 to £4,899 plus London Weighting or Senior Scientific scale, if applicable, £5,451 to £6,837 plus London Weighting N H S Designated scale 9 £4,959 to £6,025 plus London Weighting

Ring 01-205 7041 for a more detailed job description (Dr L R Hill, on Extn. 47) and for an application form (Personnel Officer, on Extn 12)—Central Public Health Laboratory, Colindale Avenue, Colindale, London, N W 9 Closing date February 15, 1979

9293(A)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOG
DIVISION OF IMMUNOLOGY

RESEARCH TECHNICIAN

for a project on new immunologi methods to detect microbial antig and antibodies Gonococci a Chlamydia are studied by radi immunoassay and mixed rever passive antiglobulin haemagglutinatio A wide variety of bacteriological a biochemical techniques are used

Qualifications needed are a degre HNC or equivalent, with laborato experience preferably in microbiolo The University offers good worki conditions in Laboratories at Ne Addenbrooke's Hospital with mode equipment and the opportunity aris to work alongside first class Scienti

Grade R6 Scale £2,959 to £3,4 (under review) First Class Pensi Scheme, etc.

Please send details in the fir instance to The Superintende Department of Pathology, Ten Court Road, Cambridge CB2 1QP

9455(A)

UNIVERSITY MARINE BIOLOGICAL STATION MILLPORT

Isle of Cumbrae, KA28 0EG

Applications are invited for

TECHNICAL POSTS

in the Grades UTS3 and UTS1

Further details and applicati forms may be obtained from th Station Secretary at the above addre to whom completed forms should returned not later than February 1979

9456(A)

MEDICAL RESEARCH COUNCIL

Radiobiology Unit,
Harwell, Oxon

A vacancy has arisen for a

RESEARCH OFFICER/ TECHNICIAN

to work on studies of damage to DNA by ionising radiation Experience in either microbiology or electron-microscopy of macromolecules would be an advantage Enthusiasm and adaptability are essential

Qualifications for Technician are H N C. or equivalent while a Research Officer would have at least two years relevant experience in addition

Salary scales are £3,261 to £4,680 for Technician and £3,582 to £4,902 for Research Officer. Assisted transport and modern hostel accommodation for single people are available

Applications, giving full personal details including the names of two referees, should be addressed to The Administrator, M.R C Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD Ref KB2

9459(A)

UNIVERSITY OF BRISTOL

DEPARTMENT OF GEOLOGY

ROCK-SECTION TECHNICIAN WANTED

Applications are invited for a person to take charge of the rock-cutting workshop The appointed person will have had several years experience in this type of work together with some educational qualifications

The position carries a salary of £3,186 to £3,720 per annum (Grade 5) (scale under review).

Applications, including the names of two referees, to The Superintendent, Department of Geology, Queens Building, University Walk, Bristol BS8 1TR To be received within two weeks of this advertisement appearing

9449(A)

UNIVERSITY OF READING

DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY

RESEARCH ASSISTANT

required as soon as possible for an S R C funded research project on the neuroendocrine control of ovulation in birds The appointment will be for a fixed period of up to two years depending upon the starting date Applicants should be graduates in physiology, pharmacology or zoology and should have a good background in neurophysiology An interest in neuroendocrinology would be an advantage Starting salary up to £3,634 per annum (under review) U S S superannuation Apply, quoting Ref MN 13A, with the names of two referees to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH

9407(A)

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR

Applications are invited for the post of LECTURER IN MATHEMATICS Applicants should have research experience and publications in Pure Mathematics, preferably in an area of TOPOLOGY

The appointment, to commence on October 1, 1979, will be to the Universities' Lecturer scale —£3,883 to £7,754 per annum

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained

Closing date for applications February 28, 1979

9412(A)

We wish to appoint someone with experience in the medical or biological sciences to head a fully established research team in our Preclinical Research Department. The work of this team is mainly concerned with gerontological and pharmacological problems.

The person appointed will have a university degree in the medical or biological sciences backed by practical experience in the fields of anatomy, pathology or brain research Importance is also attached to a knowledge of morphometry and/or stereology and to a good command of the English language.

Applicants are asked to send a short letter of application in the first instance to:

SANDOZ

Sandoz Ltd., Personnel Dept. Ref. 1200,
Postbox, 4002 Basle, Switzerland.

9321(A)

THE MEDICAL COLLEGE OF ST BARTHOLOMEW'S HOSPITAL

(University of London)

West Smithfield, London EC1A 7BE

LECTURER POSTS IN PATHOLOGY WITH AN INTEREST IN TOXICOLOGY

Applications are invited for two posts as Lecturers in Pathology, tenable for a period of three years in the first instance The posts are suitable for those seeking their M R C Path and who are likely to develop an interest in toxicology with a particular emphasis on comparative histopathology.

The first two years would be spent mainly in following the normal training schedule rotating through two or hree of the four pathology disciplines at St Bartholomew's Hospital under he supervision as appropriate of the Professors of Histopathology, Chemical Pathology, Haematology and Medical Microbiology, and partly in being introduced to the toxicology work of he Department of Health and Social Security.

After completion of the Primary examination there will be opportunities for specialisation in comparative histopathology and toxicology under the supervision of the Director of D.H S S Toxicology Laboratory at St. Bartholomew's and in the Medical College of St. Bartholomew's Hospital A further fixed term contract of two years would be offered for this purpose Candidates completing the membership examination of the Royal College of Pathologists would, in addition to opportunities in Universities, Industry and the National Health Service, be eligible to apply or specialised posts in the Medical Divisions of D H S S in the field of oxicology The promotion prospects n D H.S S are good and there would be opportunities for research, special tudy and attendance at meetings

Salary for these posts will be on the cale £4,986 to £7,393 per anum plus '354 London Allowance.

Application forms and further information may be obtained from the Dean's Office of the Medical College t the above address, and should be returned to the Dean not later than February 28, 1979 9390(A)

QUEEN ELIZABETH COLLEGE

Kensington

(University of London)

PART-TIME LECTURER

DEPARTMENT OF MATHEMATICS required in session 1979–80 only, to teach a course of operational research to third year mathematics students. Forty lectures plus some tutorials, marking and examining Fee £800

Apply to Professor W B Bonnor, Mathematics Department, Queen Elizabeth College, Campden Hill Road, London W8 7AH 9442(A)

CHELSEA COLLEGE

University of London

GRADE 2B TECHNICIAN

Required to assist in research and teaching activities within the Biophysics Section of the Basic Medical Sciences Group Experience of Biophysical/Bioengineering or Physiological techniques would be an advantage.

Salary scale (under review) £2,994 to 3,345, inclusive of London Allowance.

Enquiries and application forms om the Secretary, Biophysics Laboratory, Chelsea College, 17A Onslow Gardens, London SW7 3AL (01-589 98). 9452(A)

SOUTH AFRICA

Veterinary Science Officers

Onderstepoort

The Department of Agricultural Technical Services at Onderstepoort in South Africa, wishes to appoint additional scientific staff for the establishment of the new Foot and Mouth Disease Laboratory which will be provided with all modern facilities for research and vaccine production.

Positions are available at several levels of seniority and applicants should hold the following minimum qualifications:

State Veterinarians	BVSc (or equivalent) degree
Chief Professional Officer (Veterinary)	BSc. (Agric.) Honours (or equivalent degree)
Chief Technician (Veterinary)	Diploma in Veterinary Technology or Diploma in Microbiology

Applicants must show initiative in undertaking research work and have experience in any of the following fields:

Virology; Immunology; Vaccine production and testing; use of fermenters for the production of cells in suspension.

Experience in Foot and Mouth Disease laboratories will be a recommendation.

Commencing salary will depend on qualifications and experience and appointments will initially be under agreement for a minimum period of three years (which can be extended to five years, after which the employee will be repatriated if desired).

Among the benefits offered are free passage to South Africa, removal of a limited amount of household effects and a "settling-in" allowance.

For further information contact Johan Cloete, Civil Service Officer, South African Embassy, Trafalgar Square, London WC2N 5DP.

Tel: 839 2211 ext 141/142. 9372(A)

Readvertisement

UNIVERSITY OF OXFORD

E. P. ABRAHAM PROFESSORSHIP OF CHEMICAL PATHOLOGY

The electors intend to proceed to an election to the E P. Abraham Professorship of Chemical Pathology with effect from October 1, 1980 The stipend of the professor is at present £10,828 per annum

Applications (nine copies), naming three referees but without testimonials, should be received not later than March 26, 1979, by the Registrar, University Offices, Wellington Square, Oxford OX1 2JD, from whom further particulars may be obtained 9446(A)

POSTDOCTORAL POSITION

Research on the molecular biology of insect development, hormonal control of mRNA synthesis and in vitro transcription. Experience in nucleic acid hybridizations desired. Send resume and names of three references to. Dr S Sridhara, Department of Biochemistry, University of Mississippi Medical Center, 2500 North State Street, Jackson, Ms 39216 The University of Mississippi Medical Center offers equal opportunity in education and employment, Male/Female. 9376(A)

SURFACE PHYSICIST/ CHEMIST

Fusion energy research on plasma-wall interaction phenomenon involving atomic hydrogen chemical sputtering and surface cleaning. Uhv and surface physics/chemistry experience appropriate Appoint at Research Associate or Senior Research Associate level. Appointment for three years initially Well-qualified scientist sought. Appropriate salary available Write: Professor P. C. Stangeby, University of Toronto, Institute for Aerospace Studies, 4925 Dufferin Street, Downsview, Ontario, M3H 5T6 (416) 667-7729 9379(A)

THE UNIVERSITY OF MANCHESTER

LECTURER IN GEOLOGY

Applications are invited for this post from persons with an interest in Economic Geology, with particular reference to Ore Deposits. Initial salary range £3,883 to £4.631 per annum. Particulars and application forms (returnable by February 22) from the Registrar, The University, Manchester M13 9PL. Quote ref. 12/79/N. 9381(A)

THE UNIVERSITY OF LANCASTER

Postgraduate Research Assistant—Environmental Radioactivity

A postgraduate

RESEARCH ASSISTANT

is required for a three-year research project on the transport of artificial radionuclides from the marine to the terrestial environment The research is supported by a grant from the Natural Environment Research Council to Dr S R. Aston, of the Department of Environmental Sciences Applicants should have a good honours degree in chemistry, physics or environmental sciences, and experience in radioactive work.

Salary will be on the scale £3,884 to £4,882.

Further details may be obtained (quoting reference L105A) from the Establishment Officer, University House, Lancaster LA1 4YW, to whom applications (five copies) should be sent not later than March 16, 1979 9366(A)

UNIVERSITY OF VICTORIA
TRIUMF
Physics Department
requires
Research Associates in Intermediate Energy Physics

Two Research Associate positions are available for research in Experimental Physics at the TRIUMF 500 MeV Cyclotron. The successful applicants will be engaged in the University of Victoria research program at the Cyclotron, which includes studies of pionic and muonic X rays, rare decay modes of the pion and muon, and nucleon-nucleon scattering.

These appointments will be made initially for one year and are renewable. Salary will depend on experience, with a minimum of $15,500 per annum.

Send curriculum vitae, list of publications, and names of three referees to: **Dr. L.P. Robertson, Department of Physics, University of Victoria, Victoria, B.C. V8W 2Y2.**

UNIVERSITY OF VICTORIA

9409(A)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of LECTURER in the above Department. The person appointed will participate in teaching biochemistry to science and to medical students. The Department provides good facilities and opportunities for research. The appointment will date from October 1, 1979.

Salary on the scale for lecturers: £3,883 to £7,754 (under review) according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 83/71/D. Closing date for applications, March 12, 1979.

9367(A)

UNIVERSITY OF EDINBURGH
LECTURESHIP IN MICROBIOLOGY

Applications are invited for a newly established Lectureship in the Department of Microbiology. Applicants should have some experience or interest in applied microbiology, preferably in the area of agriculture or food.

Salary on scale £3,883 to £7,754 per annum, with superannuation benefits.

Further particulars may be obtained from the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, with whom applications by letter (five copies), including the names of three referees, should be lodged not later than February 28, 1979. Please quote Reference 1007.

9393(A)

AGRICULTURAL RESEARCH COUNCIL
Plant Physiologist

Applications are invited from Plant Physiologists who would like to join a small team of senior scientists who advise the Secretary of the Council on the scientific aspects of research administration.

The post is graded Senior Principal Scientific Officer with a **salary scale of £10,043 to £11,300 plus £465 for working in Central London.** Superannuation is under a non-contributory scheme analogous to that of the Civil Service.

Applications from candidates with a good honours degree of equivalent qualification and a successful record of research should be by letter accompanied by a full curriculum vitae and the names of two referees. Applications from candidates who applied previously will be automatically considered.

Further particulars from Miss M. J. Gard, Agricultural Research Council, 160 Great Portland Street, London W1N 6DT. Closing date March 9.

9351(A)

JUNIOR LABORATORY SCIENTIFIC OFFICER required for one year in the first instance for a research project on the biochemistry of muscular dystrophy supported by a grant from the Muscular Dystrophy Group of Great Britain. The work will involve measurement of enzyme activities and investigations of the properties of cell membranes. Some experience in handling animals is highly desirable. Salary in range £2,247 (age 18) to £2,904 or £2,370 to £3,015 according to age and qualifications, plus £354 London Weighting. Apply in writing, stating age and giving details of qualifications and experience (if any) to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. B.C.

9406(A)

FACULTY POSITIONS OPEN
College of Agriculture and Food Sciences
KING FAISAL UNIVERSITY
Hoffuf, Saudi Arabia

Inquiries invited from qualified applicants at all academic levels to teach courses in the following fields:

Agricultural Economics
Animal Science
Animal Ecology
Agronomy
Agricultural Engineering
Dairy Science
Bee Keeping
Horticulture
Agricultural Extension
Water Resources and Climatology
Plant Pathology
Home Economics and Nutrition
Poultry Science

PREREQUISITES: Masters or equivalent degree; practical and/or teaching experience. Preference will be given to Arabic speakers.

Language of instruction is English.

Positions are available starting September 1979; interviews scheduled with the Dean in the United States and Great Britain during April/May 1979.

SALARY RANGES: Gross monthly salary in Saudi riyals. Approximate annual (sterling equivalent).

RANK:

Professor SR7,195 to SR9,595 (£12,983 to £17,314). Associate Professor SR6,435 to SR8,185 (£11,612 to £14,770). Assistant Professor SR5,245 to SR7,765 (£9,466 to £14,012). Lecturer SR3,280 to SR4,530 (£5,919 to £8,174).

BENEFITS

Free furnished accommodations; Substantial educational allowance for up to four children. Air fare to and from Saudi Arabia once a year for husband, wife and two children plus 60-day paid summer holiday. No Saudi income tax.

Send complete curriculum vitae stating preference of course and names, addresses and telephone numbers of three references to:

Dr H. M. Elarosi
Dean, College of Agriculture
and Food Sciences
King Faisal University

at the appropriate addresses below:

Post Office Box 2, Sunbury-on-Thames, Middlesex TW16 5JP, England.

c/o Saudi Arabian Educational Mission, 2223 West Loop South, Suite 400, Houston, Texas 77027, U.S.A.

9399(A)

POSTDOCTORAL POSITION. Naval Research Laboratory/Georgetown University collaborative programme. Raman and Brillouin light scattering investigations into molecular conformations and viscoelasticity in polymers and biopolymers as functions of pressure and temperature. Salary $15,000 plus travel expenses. Send résumé and names of three references to R. Weiss, Department of Chemistry, Georgetown University, Washington, D.C. 20057. An equal opportunity employer.

9410(A)

UNIVERSITY OF DURHA
DEPARTMENT OF APPLIED PHYSICS AND ELECTRONIC

Applications are invited for the
of

LECTURER

in the Department of Applied Phy
and Electronics tenable from Octo
1, 1979. Candidates should have
search and/or industrial experience
the general area of microelectron
Preference will be given to those
research interests in silicon dev
and/or technology.

Salary on the scale £3,883 to £7.
(under review), plus superannua
benefits, according to age
experience.

Applications (three copies) nam
three referees should be submitted
the Registrar and Secretary, Scie
Laboratories, South Road, Durh
DH1 3LE, not later than Mon
February 26, 1979, from wh
further particulars may be obtai

9356(A

UNIVERSITY OF NOTTINGHAM
SCHOOL OF AGRICULTURE
Sutton Bonington, Loughboroug

Applications are invited for the pos

TECHNICIAN GRADE 3

within the Animal Production Sec
of the Department of Agriculure
Horticulture. The successful applic
will be required to assist in stu
of the factors controlling reproduc
in farm livestock, and the work
largely involve the radioimmunolog
assay of hormones. Previous exp
ence with such techniques is
essential. The appointee will also
responsible for the day to day runn
of the appropriate laboratory facilit

Salary in accordance with Grad
for Technicians, £2,688 to £3,060
annum.

Write or telephone the Secretary
the School (Tel: Kegworth 2386)
an application form quoting Post
79/2.

9359(A

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF PHARMAC
LECTURER IN PHARMACEUTICS

Applications are invited fr
Pharmacy Graduates for a Lectures
in Pharmaceutics. Experience in P
sical Pharmaceutics or Pharmaceut
Technology would be considered
advantage.

The initial salary will be within
range £3,883 to £7,030 per annum

Further particulars and forms
application, returnable not later t
February 16, 1979, from the S
Appointments Officer, University
Nottingham, University Park, N
tingham NG7 2RD. Ref. 660.

9360(A

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF BIOLOGY

Applications are invited for the
of Lecturer in the Department
Biology. Candidates should have
special interest in Plant Ecology
should expect to contribute fully
the general teaching in the Dep
ment.

Salary scale £3,883 to £7,754
annum plus U.S.S. Initial salary
depend on qualifications and exp
ence.

Further particulars may be obtai
from Mrs E. C. P. Sears, Staff
Section, The University, Highfi
Southampton SO9 5NH, to wh
applications (seven copies), nam
three referees should be sent, as s
as possible and not later than Ma
9, 1979. Please quote refere
number 1609/R/Na.

9362(A

UNIVERSITY OF SOUTHAMPTON
NEUROPHYSIOLOGY RESEARCH GROUP

Applications are invited for a technical appointment in the School of iochemical and Physiological Sciences assist in a current research project Mammalian Neurophysiology and europharmacology.

Applicants should possess an approiate science qualification, at least .N.C. or two science A level passes, d have relevant experience. Applicaons from graduates in Physiology d Pharmacology would be welcome.

The appointment which is to be led as soon as possible, and is for e year in the first instance with e possibility of an extension for a rther two years, will be made on lary scale £2,688 to £3,060 per num (under review), with initial lary up to £2,835. Opportunities for rther study would include part-time gistration for a higher degree if propriate.

Applications stating date of birth, alifications and experience and the mes and addresses of two referees, ould be sent to Mr C. N. Saull, The niversity, Southampton SO9 5NH, oting reference number 517/T/Na.

9361(A)

Hounslow Health District
WEST MIDDLESEX HOSPITAL
Isleworth, Middlesex
ASIC GRADE BIOCHEMIST

quired in clinical biochemistry oratory. Previous experience not ential. Wide range of techniques. luding automation and radiomune assay, providing the experice and opportunities needed to take gher qualifications.

The Department may be visited by pointment. Tel: 01-560 2121, Ext. 8. Whitley Council terms and conions of service apply.

Application form from: Mrs Pittan, Personnel Department. Tel: 560 21, Ext. 669. 9369(A)

RESEARCH ASSISTANT

Graduate required to join a team of entists investigating basic aspects cardiac biochemistry and pharcology. A training in pharmacology, chemistry or physiology and an perience in spectrophotometry, tissue tabolite analysis or organ perfusion uld be advantageous. The research it is located in the new Rayne titute and appointments will be de through St. Thomas's Hospital dical School.

Applications, together with a curulum vitae, should be sent to Dr an Manning, The Myocardial tabolism Research Laboratories, e Rayne Institute, St. Thomas's spital, London S.E.1. 9391(A)

UNIVERSITY OF OXFORD
DYSON PERRINS (ORGANIC CHEMISTRY) LABORATORY
DEPARTMENTAL ASSISTANT

Applications are invited from anic chemists of postdoctoral status the post of Department Assistant n September 1, 1979.

The salary will be on the scale 883 to £4,882 per annum according age and experience and with nbership of U.S.S.

The appointment will be for a iod not exceeding two years and successful applicant will be ected to collaborate with one of research groups and supervise ergraduate experiments.

urther particulars may be obtained n the Administrator, Dyson Perrins oratory, South Parks Road, Oxford 1 3QY, to whom applications with names of two referees should be by March 31. 9398(A)

Technician
Drug Metabolism

A vacancy exists for a Technician to work in our Drug Metabolism Department. He/she will assist in determining the metabolic fate of candidate drug compounds in both animals and man. There will also be some involvement in carrying out animal procedures.

Candidates should have O levels, HND or equivalent qualifications. Home Office licences would also be advantageous.

We are part of a large expanding international company in which salaries and job security are of the high standard to be expected.

Benefits include minimum of four weeks holiday, contributory pension scheme with free life insurance, free membership of PPP, flexible working hours, staff restaurant with luncheon vouchers, sports and social club and contribution towards removal expenses where appropriate.

Please write or telephone for an application form to: Adrian Forrest, Personnel Department, Hoechst UK Limited, Walton Manor, Walton, Milton Keynes, Bucks. Tel: Pineham 5068.

9447(A)

Hoechst

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMISTRY
RESEARCH ASSISTANT
(Ref: EST/21/79J)
and a
RESEARCH TECHNICIAN
(Ref: EST/113/79J)

Applications for the above vacancies, in a research group working on the structure and assembly of bacterial multimeric membrane proteins, are invited from suitably qualified candidates with an interest or experience in one or more of protein purification, immunological techniques and/or electron microscopy. The posts are financed from a Medical Research Council grant and are available to September 30, 1981, with the possibility of subsequent renewal.

Applicants for the Research Assistant post must have a good Honours degree in Biochemistry or a related subject and will be eligible for appointment within the range £3,384 to £3,883 per annum (under review). Applicants with appropriate O.N.C. or equivalent qualifications and at least three years' relevant experience will be eligible for appointment to the Technician post at an initial salary of £2,688 (under review).

Applications, containing full details of age, qualifications and experience and the names of two referees should be lodged as soon as possible with the Secretary, The University, Dundee DD1 4HN. Please quote appropriate reference.

9385(A)

SEA MAMMAL RESEARCH UNIT
HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER
ENERGETICS OF SEALS AND WHALES

The Unit is looking for a biologist to join a team studying the energy requirements of seals and whales. The post will involve fieldwork under rigorous conditions, including work from small boats and on remote islands. Appointment will be at the Higher Scientific Officer or Senior Scientific Officer level depending on experience, and will commence as soon as possible.

Qualifications

A degree in a biological subject plus at least three years' postgraduate experience. Candidates should have a background in appropriate laboratory techniques. Experience in the capture and handling of large mammals and the use of telemetry equipment would be advantageous.

Salary

Salary range £4,101 to £5,448 (H.S.O.) and £5,154 to £6,898 (S.S.O.). Starting pay according to qualifications and experience. Non-contributory superannuation scheme.

The staff of the Council are not Civil Servants, but their pay and conditions of service are similar to those in the Civil Service.

For application form and further particulars write (quoting reference SM3) to SEA MAMMAL RESEARCH UNIT, c/o BRITISH ANTARCTIC SURVEY, MADINGLEY ROAD, CAMBRIDGE CB3 0ET, CAMBS.

Closing date: February 26, 1979.

NATURAL ENVIRONMENT RESEARCH COUNCIL

9354(A)

UNIVERSITY OF BATH
SCHOOL OF PHARMACY AND PHARMACOLOGY
LECTURER IN PHARMACEUTICAL ANALYSIS

Applications are invited from suitably qualified persons for the above post. The successful applicant will be expected to contribute to the teaching of B.Pharm. and B.Sc. (Pharmacology) undergraduate courses and be actively interested in research in a branch of Pharmaceutical Analysis.

Application forms and further details available from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting Reference No. 79/17/N.

9373(A)

UNIVERSITY OF ABERDEEN
DEPARTMENT OF ZOOLOGY
RESEARCH ASSISTANT

Applications are invited for the above post, funded by World Health Organisation, to study amino acid transport and protein synthesis in a blood-dwelling parasite. Candidates should possess an honours degree in Biochemistry, Zoology or Parasitology.

The appointment will be for 30 months, to commence as soon as possible. Salary (within Range 1B) £3,384 to £3,883 per annum with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by February 23, 1979, quoting reference LC1. 9374(A)

DEAN
ONTARIO VETERINARY COLLEGE
UNIVERSITY OF GUELPH

Applications and nominations are invited for the position of Dean of the Ontario Veterinary College, which will become vacant on December 31, 1979 The Ontario Veterinary College is the oldest college of veterinary medicine in Canada with a complement of 105. The College is administered through the Dean with the assistance of two Associate Deans.

It is expected that candidates will hold the D.V.M or equivalent veterinary qualifications preferably with further advanced training Considerable experience in teaching, research and administration in an academic environment will also be expected.

The successful applicant will also hold an academic appointment in the appropriate department of the Ontario Veterinary College.

Applications and nominations will be accepted until April 1, 1979, or until position is filled, and should be submitted to · Dr. H C Clark, Vice-President, Academic, University of Guelph, Guelph, Ontario, Canada, N1G 2Wl.

Position subject to final budgetary approval

9387(A)

UNIVERSITY OF CRETE

The Departments of Mathematics and Physics are having openings for visiting appointments at all ranks Greek-speaking mathematicians and physicists are invited to apply Applications for regular appointments will be formally solicited soon.

All correspondence, including letters of recommendation, should be sent by the end of April 1979 to Ms Sophia Politi, University of Crete, Solonos 71, Athens 143, Greece 9383(A)

UNIVERSITY OF BIRMINGHAM
Dental School
DEPARTMENT OF ORAL PATHOLOGY
RESEARCH FELLOW

Postdoctoral Research Fellow required to continue a project into the development of *in vitro* methods for testing the toxicity of dental materials Previous experience of cell culture and quantitative cytochemical techniques would be an advantage, but is not essential The project is for three years and would be carried out in parallel with other *in vivo* projects currently in progress

Salary on the scale £3,883 to £6,555, plus superannuation

Further particulars available from the Assistant Registrar, The Dental School, St Chad's Queensway, Birmingham B4 6NN, to whom applications, including a full curriculum vitae and the names of three referees should be sent by February 28, 1979

9370(A)

University of London
READERSHIP IN PHYSIOLOGY AT QUEEN ELIZABETH COLLEGE

The Senate invites applications for the above Readership to be held at the Department of Physiology (Head of Department Professor D L Yudilevitch, M D) The Reader will be an experienced physiologist of proven research ability The Department has been expanding its research activities in the past few years and is now well-equipped for studies in its current interests Queen Elizabeth College is located in Kensington in a pleasant neighbourhood near Holland Park

The successful candidate will be expected to develop his/her own research, supervise postgraduate research students and to contribute to undergraduate teaching

Applications (10 copies) must be received not later than March 30, 1979, by the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, from whom further particulars (including the salary scale) should first be obtained

9451(A)

Pharmacologist
Safety of Medicines

The Pharmaceuticals Division of ICI requires a graduate pharmacologist, male or female, interested in pharmacodynamics and mode of action studies Considerable experience and knowledge of the pharmaceutical industry would be an advantage

The successful candidate is likely to be in the age range 25-35, and will be expected to contribute to a multidisciplinary team working on the safety of novel pharmaceuticals, and to work closely with skilled technical staff, veterinary and other graduate staff The main responsibility of the post will be to complete the studies of biochemical and physiological effects of drugs and their mechanism of action

Salary and conditions of service will be attractive and the position is based at our headquarters in pleasant countryside in Cheshire with good recreational and residential facilities

Please write with your career details to

ICI

Mr M F Losse,
Personnel Officer,
ICI Pharmaceuticals Divison,
Mereside, Alderley Park,
Nr Macclesfield,
Cheshire

9445(A)

SEALE-HAYNE COLLEGE
Newton Abbot, Devon
LECTURER IN CROP PRODUCTION

Applications are invited from su ably qualified graduates with teachin research or industrial experienc Duties include teaching degree a diploma students

Salary based on Scales of Salari for Teachers in Establishments f Further Education, England a Wales, Lecturer Grade II (£4,101 £6,558 per annum)

Applications to and further pa ticulars from The Principal, Seal Hayne College, Newton Abbot, Devo TQ12 6NQ 9426(A)

UNIVERSITY OF EXETER
GEOGRAPHY DEPARTMENT

Applications are invited for the po of Grade V Laboratory Technician take overall responsibility for th physical laboratories of the Depa ment of Geography, to develop th chemical analysis of soils and se ments, and to extend, advise and gi instruction on laboratory procedur

A background in environmental/ chemistry would be an advantage.

Applicants should hold H N C equivalent qualification at least, a have appropriate experience in t work

Salary £3,186 to £3,720 per annu (under review)

Further details can be obtain from Miss Doreen Watson, to who applications, stating age, qualificatio and experience together with th names of two referees should be se by March 2, 1979 Please quo reference number 5111 9437(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BIOCHEMISTRY
TECHNICIAN (ELECTRO MICROSCOPE)

To operate a J E M T7 electr microscope and associated equipme which provides a service to seve research groups Candidates shou possess H N C or equivalent minimu qualification and several years expe ence but applications from candida with lower qualifications will be co sidered Salary on a scale up to £3,7 p a according to qualifications a experience (under review)

Application forms may be obtain from The Registrar, The Universi PO Box 147, Liverpool L69 3B Quote Ref: RV/475/N 9438(A)

UNIVERSITY COLLEGE OF NORTH WALES
Bangor

Applications are invited for the post of **RESEARCH ASSISTANT** to undertake a project sponsored by the Welsh Office to investigate the biodegradation of crude oil wastes

The work will be carried out in the Department of Biochemistry and Soil Science under the supervision of Dr G D Floodgate and Dr P A Williams

The appointment, to commence as soon as possible, will be for one year at a salary of £3,38 per annum.

Applications (two copies), givin details of qualifications and experience, together with the name and addresses of three referees should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor Gwynedd LL57 2DG, from whom further particulars may be obtained.

Closing date for applications February 14, 1979 9357(A)

UNIVERSITY OF DAR ES SALAAM

Tanzania

Applications are invited for the following posts in the

DEPARTMENT OF PHYSICS

1. SENIOR LECTURER/ ECTURER IN GEOPHYSICS

Applicants should hold a Ph D or uivalent in Physics with a bias in ophysics The appointee should be dy to run a geomagnetic observatory uated on the campus

2. SENIOR LECTURER/ LECTURER IN ELECTRONICS

Applicants should hold a Ph D or uivalent in Electronics The ap- ntee will be responsible for organis- and teaching undergraduate and tgraduate courses He/she will also rticipate in the development super- ion of postgraduate research in the a of specialisation

Salary scales Senior Lecturer 1,980 to £T2,480 per annum Lec- rer £T1,620 to £T1,980 per annum clusive of 50 per cent expatriation owance) (£T1=£1 33 sterling) The itish Government may supplement aries in range £4,356 to £4,782 per num (sterling) for married ap- intees and £2,754 to £3,018 per num (sterling) for single appointees viewed annually and normally free all tax) and provide children's edu- ion allowances and holiday visit sages Family passages, F S S U nnial overseas leave

Detailed applications (two copies) th curriculum vitae and naming ee referees to be sent direct to ief Academic Officer, University of r es Salaam, PO Box 35091, Dar es laam, Tanzania, by March 14, 1979 plicants resident in the U K should o send one copy to Inter-University uncil, 90/91 Tottenham Court, ad, London WIP 0DT Further tails may be obtained from either dress 9415(A)

UNIVERSITY OF DAR ES SALAAM

Tanzania

Applications are invited for the post of

ASSOCIATE PROFESSOR/ SENIOR LECTURER/ LECTURER IN GEOLOGY

Applications are invited from those o can teach two or more of the lowing courses Precambrian Ge- gy, Economic Geology, Mining ology, Geology of Fuels, Geo- emistry, Mineralogy, and Petrology ndidates should have a Ph D in ology with at least two years' teach- experience at a University or an titution of equivalent status Can- ates with long professional experi- e and without the aforementioned idemic qualifications also will be isidered

Salary scales Associate Professor 2,380 to £T2,580 per annum Senior cturer £T1,980 to £T2,480 per num Lecturer £T1,620 to £T1,980 annum (exclusive of 50 per cent patriation allowance) (£T1=£1 33 rling) The British Government may plement salaries in range £4,356 to 220 per annum (sterling) for married pointees and £2,754 to £3,300 per num (sterling) for single appointees viewed annually and normally free all tax) and provide children's edu- ion allowances and holiday visit sages Family passages, F S S U, nnial overseas leave

Detailed applications (two copies) h curriculum vitae and naming ee referees to be sent direct to ief Academic Officer, University of r es Salaam, PO Box 35091, Dar es aam, Tanzania, by March 14, 1979 plicants resident in the U K should send one copy to Inter-Universit ncil, 90/91 Tottenham Court d, London WIP 0DT Further ils may be obtained from either ress 9416(A)

Institute of Terrestrial Ecology

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER PLANT ECOLOGIST

A plant ecologist at Higher Scientific Officer/Senior Scientific Officer level is required at the Institute's station at Monks Wood, near Huntingdon

Applicants should have an interest in the experimental management of lowland heaths and will have responsibility for an important research contract concerned with the control of scrub and bracken In addition to contract work there will be opportunities for the development of other studies of vegetation change

Qualifications

Candidates should have a good honours degree in ecology, biology, agriculture or an allied subject and at least two years' relevant postgraduate experience.

Salary and conditions of service

The salary will be within the following scales

H S O. £4,101 to £5,448 per annum,

S.S O £5,154 to £6,898 per annum with 22 days paid annual leave plus 10½ days public and privilege holidays

Starting pay according to age, qualifications and experience

There is a non-contributory superannuation scheme

The Natural Environment Research Council is not a Government Department but conditions of service are similar to those of the Civil Service

Application forms and further details are available from

Establishments Section
Institute of Terrestrial Ecology
68 Hills Road
Cambridge
CB2 1LA (0223 69745)

Please quote reference 182

Closing date for applications is February 16, 1979

NATURAL ENVIRONMENT RESEARCH COUNCIL

9355(A)

BRITISH ANTARCTIC SURVEY—CAMBRIDGE HIGHER SCIENTIFIC OFFICER

The Atmospheric Sciences Division of the B A.S requires a Higher Scientific Officer to be responsible for the reduction and publication of data collected by the Survey's three geomagnetic observatories in Antarctica In addition the duties would include giving support to research staff using geomagnetic data, and maintenance of equipment Experience in computing is desirable

As the person appointed will be required to visit Antarctica in the Austral summer for six months about once every three years, applicants should be physically fit and male

Qualifications A degree, H N C or equivalent in an appropriate subject plus at least five years appropriate experience.

Salary and conditions In the scale £4,101 to £5,448 per annum—the starting salary may be above the minimum according to age and experience

Additional allowances are payable for Antarctic service during which time messing and polar clothing are free.

Staff of the N E R C are not Civil Servants, but their pay and conditions of service are similar to those of the Civil Service There is a non-contributory superannuation scheme

For application forms please write (quoting reference BAS/003) to

The Establishment Officer,
British Antarctic Survey
Madingley Road,
Cambridge CB3 0ET
Closing date February 26, 1979

NATIONAL ENVIRONMENT RESEARCH COUNCIL

9353(A)

CSIRO AUSTRALIA

Research Scientist

(2 Positions)

Division of Mathematics and Statistics

Sydney or Melbourne

CSIRO has a broad charter for research into primary and secondary industry areas The Organization has approximately 7,000 employees—2,300 of whom are research and professional scientists—located in Divisions and Sections throughout Australia

General: The Organization's Division of Mathematics and Statistics has 104 staff, of whom over 70% are professional scientists, located at centres and laboratories in all capital cities and some major country towns

The Division's research in mathematics and statistics includes various aspects of fluid mechanics, statistical mechanics, optimisation, computational mathematics, distribution theory, multivariate analysis, stochastic processes, geostatistics, statistical inference and experimental design Application of this research to agricultural, biological, environmental, physical and industrial investigations currently includes the design and analysis of experiments and the modelling of ecological, physical, agricultural, and climatological systems Research is also being extended into industrial processes and mineral survey.

Professional scientists of the Division advise on the mathematical modelling and statistical analysis of investigations undertaken by other Divisions of CSIRO The Division now wishes to appoint to its Sydney and Melbourne groups, two experienced scientists at the Senior Research Scientist–Principal Research Scientist level

Location: Following an initial period of one to two months in Canberra, the two appointees will be located in Sydney and Melbourne respectively

Duties. To conduct methodological research in statistics and/or probability theory, and to advise scientists in other CSIRO Divisions on the planning of and interpretation of experiments and the use of mathematical and statistical methods The appointee would be expected to provide leadership for an appropriate sub-group within the Division of Mathematics and Statistics

Qualifications· A Ph.D. degree or equivalent qualification, with extensive research experience and an established international reputation in a relevant field Consulting experience would be an advantage

Salary: Appointment will be made within the salary range of Senior Research Scientist–Principal Research Scientist. $A19,572 to $A26,368 p a Senior scientists with appropriate research experience may be considered for appointment at a higher level

Tenure: Indefinite with Superannuation benefits

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 440/301, should reach —

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 28th FEBRUARY, 1979

Applications in U S A and Canada should be sent to —
The Counsellor Scientific, Embassy of Australia,
1601 Massachusetts Avenue, N W, Washington, D C, 20036,
U S A

9440(A)

POSTDOCTORAL POSITION

available immediately for research on membrane phenomena associated with mediator release in allergy Applicants should have a background in immunochemistry or biochemistry Send curriculum vitae and three letters of recommendation to Dr A Froese, Dept of Immunology, University of Manitoba, 730 William Ave Winnipeg, Manitoba, CANADA R3E OW3 9429(A)

University of London

CHAIR OF AGRICULTURAL BOTANY AT WYE COLLEGE

The Senate invites applications for the above Chair

Applications (10 copies) should be received by the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained Closing date March 2.

A9416(A)

BRITISH ANTARCTIC SURVEY

The British Antarctic Survey have vacancies for Contract Staff on limited period appointments. All the posts involve visits to Antarctica for varying periods.

GEOLOGISTS (3 POSTS)

(ES 1/79) GEOLOGIST required to work in the Mineralogy, Petrology and Geochemistry Section of the Survey on plutonism of the Antarctic Peninsula. This project is a study of the plutonic rocks in certain areas of the Antarctic Peninsula over two summer field seasons. Some basic mapping will be required although many of the plutonic rock areas have already been studied. Geochemical sampling and subsequent analysis to determine magmatic and regional evolutionary trends will form a major part of the programme.

(ES 2/79) An experienced metamorphic petrologist/structural GEOLOGIST is required to work on the Pre-Middle Jurassic metamorphic complex of the Antarctic Peninsula. The successful candidate will undertake two Antarctic summer field seasons, the first being spent in Palmer Land examining areas previously mapped only in reconnaissance detail. During the second season the person will be working in a collaborative US/BAS study of the tectonic relationship between East and West Antarctica.

(ES 3/79) GEOLOGIST required to study the Jurassic palaeoecology and stratigraphy of the northern Antarctic Peninsula. The project will be primarily concerned with the plant-bearing sequences; field work is planned to document accurately these deposits with a view to assessing their significance The project will involve one summer field season.

Posts 1/79 and 3/79 to commence beginning of July, 1979.

Post 2/79 is available from May 1979 and must be taken up by early July.

Qualifications for all three posts· Minimum Upper Second Class Honours degree but preference will be given to applicants holding higher qualifications or relevant research experience especially for ES 2/79 and ES 3/79.

GLACIOLOGY SECTION (2 POSTS)

(ES 4/79) GLACIOLOGIST/OCEANOGRAPHER. A person is required to assist in the examination of conditions affecting the melting of ice at the bottom of ice shelves. Additionally, oceanographic measurements of current flow, salinity and temperature profiles, and the collection of seawater samples are undertaken.

Two visits to the Antarctic would be made within the first three years employment Qualifications should be a minimum of a 2(1) Honours Degree is physics, geophysics, oceanography, chemistry or engineering, and should include practical skills.

To commence beginning of July, 1979.

(ES 5/79) GLACIOLOGIST is required to assist in the development of radio echo equipment and the interpretation of results. Airborne surveys of ice thickness will be carried out in 1980/81; the person appointed would help gather data in the Antarctic and then reduce this data in Cambridge. The 1979/80 season will be spent carrying out related experiments, but based on the glacier surface, studying ice shelves and ice sheets to help understand their dynamic behaviour.

The person appointed should have qualifications in electronics with mathematical knowledge, or have a degree in applied maths or physics and a strong interest in electronics.

To commence beginning of July, 1979.

CHEMISTS (2 POSTS)

(LS 1/79) FRESHWATER CHEMIST to study the chemical relationships involved in nutrient cycling in maritime Antarctic lakes on Signy Island, South Orkney Islands. The proposed study is part of a comprehensive programme of limnological research Laboratory facilities at the Signy research station are good and there is a teleprinter link with headquarters and the Project Leader in the UK.

The individual selected will receive up to one year's training at the research laboratories, Cambridge and the Freshwater Biological Association, Windermere. Approximately 2½ years will be spent in Antarctica and one year will be spent writing up the work in Cambridge.

Applicants should have at least a First or Upper Second Class Honours degree. SCUBA diving experience is desirable but not essential as professional training will be given. Post available from July 1979 and must be taken up by September.

(ES 6/79) A CHEMIST is required to join a small team analysing trace impurities trapped in Antarctic snow. The aim is to determine changes in airborne pollution reaching the polar ice cap during historical time and to interpret palaeoclimatic variations from microchemical evidence contained in ice cores.

Following a year spent in the laboratory the successful applicant will undertake a summer field season in the Antarctic Peninsula collecting samples and carrying out some in situ analyses, which will involve an absence from the UK of about six months.

In the first instance the appointment is for a period of four years commencing June/July 1979.

Minimum qualifications are 2nd Class Honours Degree in Chemistry or Joint Honours Degree.

ATMOSPHERIC SCIENCES DIVISION

(AS 1/79) PHYSICIST/METEOROLOGISTS (6 Posts) and (AS 3/79) METEOROLOGISTS (2 Posts) to work at the Survey's observatories in Antarctica. Posts (AS 1/79) carry special responsibility for either ozone and radiation measurements, or geomagnetic and seismological programmes.

Successful candidates will be part of a combined meteorology/observatory physics team which is responsible for surface and upper air meteorology, observations of sea ice movement and snow accumulation.

Applicants should have a degree in physics or meteorology, or relevant HND or HNC qualifications. Preference will be given to candidates with practical electronic experience.

(AS 2/79) PHYSICISTS/ELECTRONIC ENGINEERS (2 Posts) to carry out studies of the ionosphere in Antarctica The basic work consists of operating and maintaining a variety of ionospheric remote sending experiments and interpreting the results. Applicants should have a background in either electronics with an interest in atmospheric sciences or in physics with electronic experience They should be prepared to work without supervision and be capable of improvisation in adverse situations

Successful applicants will commence training in July prior to sailing to Antarctica in October and must be prepared to spend 30-33 months continuous service overseas.

BRITISH ANTARCTIC SURVEY

BIOLOGICAL OPPORTUNITIES (6 POSTS)

Offshore Biological Programme

The British Antarctic Survey is engaged in a major offshore biological programme in the waters of the Southern Ocean around South Georgia.
About 3 months of each year will be spent at sea in active sampling.

(LS 5/79 a) KRILL PHYSIOLOGIST

The zooplankton team of the Offshore Biological Programme requires a physiologist to study the energetics of Antarctic Krill (*Euphausia superba*).

The successful applicant will be expected to undertake a research programme involving a significant amount of field work centred at the Survey's base on South Georgia and making use of the laboratory and aquarium facilities there.
Qualifications: PhD or minimum Upper Second Class with strong bias towards marine invertebrate physiology.

Appointment from 1 June 1979.

(LS 5/79 b) FISH PHYSIOLOGIST

A fish physiologist is required to work on the growth and development of an Antarctic group of fish (the Nototheniidae).

Most of the field work, which will probably extend over three Antarctic seasons, will be done at the Survey's base at Signy Island On returning to the UK, up to two years will be available for writing up the work.

Qualifications: PhD or minimum Upper Second Class, preferably with emphasis in fish physiology. SCUBA diving experience is desirable but not essential, as training will be given in this country.

Appointment from 1 June 1979.

(LS 5/79 c) ASSISTANT BIOLOGIST FOR ZOOPLANKTON TEAM

The Zooplankton Team of the Offshore Biological Programme requires a biological assistant to help with the sampling, sorting, identification and curation of zooplankton samples

The successful applicant will be expected to participate in the field sampling programme around South Georgia and in the Scotia Sea as well as in sorting and identifying the samples at the Institute Headquarters in Cambridge

Qualifications: 2 'A' levels or Pass degree, with zoology as main subject. Some experience of working at sea an advantage.

Appointment from 1 September 1979.

(LS 5/79 d) CHEMISTRY TECHNICIAN (Hydrology/Primary Production Team)

A chemistry technician is required to assist with hydrological and primary production studies The work mainly involves the automated photometric analysis of nutrient chemicals including trace elements, and measurements of primary productivity using radioisotope fixation techniques. The individual selected will be based at the British Antarctic Survey research laboratories in Cambridge and will spend several months each year at sea on RRS JOHN BISCOE.

Qualifications: Applicants must have at least an HNC in chemistry. Experience with chemical auto-analysers and atomic absorption spectrophotometers is desirable but not essential as training will be given.
Appointment from 1 September 1979.

TERRESTRIAL BIOLOGICAL PROJECTS

(LS 4/79) MAMMALOGIST to study the extent and nature of Antarctic fur seal, *Arctocephalus gazella*, pup mortality on land, to investigate any relationship between mortality and density of breeding fur seals and to evaluate the role of mother-pup behaviour in such circumstances. The successful applicant will work at Cambridge for 6 months before leaving to work at South Georgia for three full field seasons commencing November 1979 and he will overwinter during 1980. He will be supported by a field assistant. It is planned that he will return to Cambridge for the 1981 Antarctic winter, returning to South Georgia for the third summer field season Thereafter he will be employed for two years in Cambridge to analyse and write up the results of his field work.

Qualifications: At least an Upper Second Class Honours degree in zoology coupled with appropriate field work or postgraduate experience.

The post is available from 1st May, 1979 and must be taken up by early July.

(LS 6/79) Vacancy for a TERRESTRIAL BIOLOGICAL ASSISTANT/TECHNICIAN to work at South Georgia or Signy Island. He will assist with current terrestrial field programmes and will maintain routine environmental monitoring programmes, equipment and biological stores. The successful candidate will undertake a period of inital training in Cambridge and assist with the preparation and dispatch of equipment for the succeeding season's terrestrial research programme.

Qualifications: Applicants should hold appropriate technical or scientific qualifications and have established experience in relevant techniques. A suitable Honours, Pass Degree, HNC or appropriate Laboratory Technician qualifications are acceptable.

Appointment from 1 September 1979. At first the individual selected will be based in Cambridge. Approximately 2½ years will be spent in the field after sailing for the Antarctic in October 1979.

Salary: For posts ES 1/79, ES 2/79, ES 3/79, ES 4/79, ES 5/79, ES 6/79 and LS 1/79 from £3689 depending on qualifications and experience.

For posts AS 1/79, AS 2/79 and AS 3/79 from £2890 depending on qualifications and experience, plus a supplement of £444.60 for periods of service in Antarctica.

For posts LS 4/79, LS 5/79a and LS 5/79b from £3689 depending on qualifications and experience.

For posts LS 5/79c, LS 5/79d and LS 6/79 from £2890 depending on qualifications and experience, plus a supplement of £444 60 for periods of service in Antarctica.

Subject to satisfactory service, Officers are eligible for a terminal payment equal to 10% of selary for service in the Antarctic.

Tax for wintering personnel is low (British Antarctic Territory or Falkland Island Dependencies rates).

Quarters, clothing, messing and canteen stores are provided free of charge while Officers are serving in the Antarctic.

ELIGIBILITY· Applicants should be male, single and aged between 21 and 35
Applications will be considered from students who expect to graduate in 1979.

For further information and an application form, please write (quoting post reference number) giving full details of qualifications and experience to:

The Establishment Officer, British Antarctic Survey, Madingley Road, Cambridge CB3 0ET.
Closing Date: - 16th February 1979.

BIOLOGISTS

...to help develop natural history exhibitions

The British Museum (Natural History) is an institution for taxonomic research and has world wide collections of animals, plants and minerals. The Department of Public Services is responsible for the dissemination of information through educational conferences, written publications and exhibitions.

As members of a team within the Department, the successful candidates will work with designers, script-writers and educational technologists on the development of new natural history exhibitions The traditional display is being replaced by a completely new scheme designed to present an integrated view of modern biology. The biologists appointed will take responsibility for the organisation and presentation of subject matter and will maintain a close liaison with designers to specify the content and structure of exhibits.

Candidates, normally aged under 27, should have an honours degree or equivalent in biology, zoology, botany or other closely related discipline. The ability to work to strict deadlines is essential.

The appointments will be for 3 years, although subsequent permanent appointment is a possibility.

Salary in the range £3300—£4880 with starting salary according to qualifications and experience.

For further details and an application form (to be returned by 16 February 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours)
Please quote ref: S/5052.

BRITISH MUSEUM (NATURAL HISTORY)

9368(A)

IMMUNOLOGIST/ VIROLOGIST

Two positions are available immediately at the Assistant Professor level for a Cellular Immunologist and a Virologist to work in the Bovine Leukemia Research Unit Applicants should have a Ph D or D.V M. degree with post-graduate training in the appropriate discipline.

Qualified applicants should send a résumé, copies of representative publications and the names of three people who may be contacted for references to: Dr Jorge F Ferrer, Chief, Section of Viral Oncology, University of Pennsylvania School of Veterinary Medicine, New Bolton Center, R.D 1, Kennett Square, Pa. 19348 9298(A)

INSTITUTE OF DERMATOLOGY

(University of London)

TECHNICIAN

required to join the research staff, currently investigating the role of pharmacological mediators of inflammation in human skin disease The successful candidate will undertake analysis of biological samples by radioimmunoassay techniques

Applications, giving full details of age, experience, qualifications and the names of two referees, should be sent to: Dr N A. Plummer, Institute of Dermatology, Homerton Grove, London E9 6BX. 9384(A)

ROYAL ALEXANDRA HOSPITAL FOR CHILDREN

Camperdown, N.S.W Australia

BIOCHEMIST

Applications are invited for the new position of Biochemist (Principal Scientific Officer) within the Department of Biochemistry in the Hospital's Institute of Pathology

The Hospital is a 400 bed Paediatric Teaching Hospital of the University of Sydney and offers a comprehensive range of clinical and laboratory services

The new appointee will assist the present Biochemist (Dr L Webster) in the routine work of the Department and will have special responsibilities in the development of new tests and techniques in the area of clinical paediatric chemistry. He/she will closely liaise with the Hospital's clinical staff and participate in the training programme of clinical and laboratory staff.

Applicants should hold a Ph D degree in Biochemistry or its equivalent, previous experience in clinical chemistry would be an advantage

Salary range $A21,049 to $A26,145 per annum.

Further information is available from Dr R K Oates, Director of Medical Services

Applications including the names of two referees should be addressed to the undersigned as soon as possible
J S Yu, General Superintendent

9283(A)

UNIVERSITY OF TORONTO

Physiology/Biochemistry

Applications are invited for two research appointments in the general area of gastrointestinal physiology, anaerobiasis and carbohydrate/protein intermediary metabolism

(a) Professional Associate Appropriate doctorate and postdoctoral experience required Initial appointment up to 5 years, renewable Salary scale floor $16,000

(b) Postdoctoral Fellow Two-year appointment Initial Salary in range $11,000 to $12,000

Applications and the names of two referees to

Professor D F Mettrick,
Chairman,
Department of Zoology,
University of Toronto,
Toronto, M5S 1A1, Canada

9327(A)

THAMES POLYTECHNIC

SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH IN MICROBIAL GENETICS

Applications are invited from good honours graduates for the post of M R C research assistant to Dr A Vivian to work on the genetics of *Acinetobacter calcoaceticus.* This project is funded by the Medical Research Council and the successful applicant will be registered with the C N A A for a higher degree

Salary £3,087 to £3,261 including London allowance.

Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by February 13, 1979

9389(A)

RESEARCH ASSISTANT

EQUINE REPRODUCTION

Keen and bright young person required to join active research group studying endocrinological and immunological aspects of reproduction in the horse Applicants should have a good B Sc degree or A I M L.S , H N C or similar technical qualifications and laboratory experience Knowledge of tissue culture, immunological, hormone assay techniques an advantage but not essential

Salary within the A R C Scientific Officer scale. (£2,839 to £4,415)

Apply in writing to Dr W R Allen, Thoroughbred Breeders' Association, Equine Fertility Unit, Agricultural Research Council, Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ 9392(A)

CITY OF LONDON POLYTECHNIC

DEPARTMENT OF BIOLOGICAL SCIENCES

MICROBIOLOGY TECHNICIAN (GRADE 4)

An experienced technician is required to provide a complete microbiological service for teaching and research. Applicants should hold the City and Guilds 735 Part II Certificate or an appropriate H.N C

Salary Grade 4 is on scale £3,441 to £3,891 inclusive of London Weighting.

Further details and application forms can be obtained from the Secretary, Department of Biological Sciences, Old Castle Street, London E1 7NT. 9352(A)

UNIVERSITY MUSEUM OXFORD

CURATORIAL ASSISTAN

(Mineralogy)

It is proposed to appoint for
period of three years an Assistant
the Curator of the Mineralogi
Collections

Salary will be on academic-rela
scale £3,384 to £4,466 per ann
(under review), according to qualifi
tions and experience, with membersh
of U S S

The work will include assistan
with the design and execution
exhibits, the identification of miner
routine accession and curation
minerals

The successful candidate is expect
to hold a degree in geology and
have some skills in mineral recog
tion and/or museum display work.

Applications, including *curriculi
vitae* and the names and addresses
two referees, should be received
later than February 20, 1979, by
F B Atkins, University Museu
Parks Road, Oxford OX1 3PW, fr
whom further particulars may
obtained 9454(A)

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF ZOOLOGY

S.R C. POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for
research post to help in a new proj
on neuronal variability in the p
thenogenetic fish *Poecilia formo*
The project has several aspects
volving behaviour, embryology, neu
physiology and anatomy, and wh
neurobiologists would be welcome
apply, such a background is
necessary The applicant should ha
completed or be about to complete
Ph.D , and be competent in d
analysis The department is w
equipped, and work related to the p
ject is being carried out in the depa
ment and elsewhere in the univers
Salary in the range £4,382 to £4,8
Superannuation The appointment
tenable for up to three years co
mencing October 1979 Applicatio
including full curriculum vitae a
the names of two referees should
sent to Professor D M Guthr
Department of Zoology, The Univ
sity, Manchester M13 9PL, from wh
further particulars can be obtain
by March 15 Quote ref 16/79/N
9358(A)

THE QUEEN'S UNIVERSITY OF BELFAST

RESEARCH APPOINTMENT

DEPARTMENT OF AGRICULTURAL BOTANY

Applications are invited from graduates, preferably with postgraduate experience, to continue a well established programme of breeding in amenity grass species being carried out at the Plant Breeding Station, Loughgall, and aimed at the production of new cultivars tolerant to grass-killing herbicides The appointment will be initially till September 30, 1980 but may be renewed Salary scale (under review) £3,384 to £6,555, initial placing depending on age, qualifications and experience

Curriculum vitae and the names and addresses of two referees should be sent to the Personnel Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland Closing date February 26, 1979 9424(A)

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)

LECTURESHIP IN FOOD SCIENCE

Applications are invited for the post Lecturer in Food Science Appli- its will be expected to have an propriate academic background, pre- ably with research experience in an a of Food Science The successful plicant will be required to teach ne unit courses in Food Science to ferent years of the degree course i to undertake research External ntracts will be encouraged Indus- il or other relevant experience uld be an advantage and placing on salary scale will reflect both alifications and experience

Salary scale £3,909 to £7,754 per num plus London allowance of 0 per annum

Apply to the College Secretary, een Elizabeth College, Campden il Road, London W8 7AH, from om further particulars and an appli- ion form may be obtained Closing e: February 28, 1979 9418(A)

AUSTRALIAN NATIONAL UNIVERSITY
RESEARCH SCHOOL OF EARTH SCIENCES
RESEARCH FELLOW IN ELECTRON MICROSCOPY

Applications for this post are in- ed from persons trained in electron croscopy, whose background is in ctural geology, physics or materials ence The successful applicant will expected to carry out research in ormation structures or other defect uctures in minerals and rocks, and be generally responsible for the OL 200Kv STEM electron micro- pe and its use in the rock defor- tion group headed by Dr M S erson There will be opportunity collaboration in research projects experimental rock deformation at h temperatures or in other research hin the School. Further details ut the Research School and the t are obtainable from the Academic gistrar of the University

Appointment will be for three years the first instance with the possibility reappointment to five years Com- ncing salary will be in accordance h qualifications and experience hin the range $A15,786 to $A20,606 annum Current exchange rates 58p $US1 14

This post was advertised earlier with closing date of October 31, 1978 plications already received will be onsidered unless candidates indicate t they are no longer interested

LOSING DATE March 9, 1979.

Reasonable appointment expenses paid Superannuation benefits are ilable for applicants who are gible to contribute. Assistance with sing is provided for an appointee m outside Canberra

The University reserve the right not make an appointment or to make appointment by invitation at any e

rospective applicants should obtain ther particulars from the Associ- n of Commonwealth Universities pts), 36 Gordon Square, London 1H 0PF 9433(A)

IMPERIAL COLLEGE
RESEARCH ASSISTANT

ostdoctoral Agricultural Scientist uired for key post in research team king on trace elements in soils in ation to agriculture Applicant uld hold good degree in agriculture soil science and preferably have ie experience of geology

tarting salary according to age and erience in the 'range £4,359 to 47 (including London Allowance) i USS

pplications to Dr I Thornton, lied Geochemistry Group, Depart- t of Geology, Imperial College, don SW7 9435(A)

Statistician

Applications are invited for the post of Statistician in the Central Medical Group of ICI Many of the non-medical skills which have become essential components of a modern occupational health service reside in this group The statistician will bring further expertise in statistical and computational methods and his/her training in logical analysis will enable him/her to make a contribution to collaborative projects

Duties will include the design, supervision and analysis of specific epidemiological studies and research into statistical and modelling techniques to clarify the role of this work in industry

The person appointed will have a degree in or with statistics, preferably with a post-graduate qualification in medical statistics and two years practical experience

The job is based in Wilmslow in Cheshire but will involve contact with people throughout ICI The starting salary, depending on experience and qualifications, will be about £5500 The Company operates Profit Sharing, Productivity and contributory Pension schemes

Write for an application form to

ICI

S D Kemp,
Personnel Officer,
Head Office
Services Department,
P O Box 3, Fulshaw Hall,
Wilmslow, Cheshire
SK9 1QB

9405(A)

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF NATURAL PHILOSOPHY
PROFESSOR OF EXPERIMENTAL PHYSICS

Applications are invited from appropriately qualified and experienced candidates for appointment to the newly established Professorship in Experimental Physics which will involve a period of years as Chairman of the Department of Natural Philosophy Applicants should have experience in one or more areas of Experimental Physics and be capable of directing postgraduate teaching and research

The post will be remunerated within the Professorial range for universities with U S S benefits

Application forms and further particulars (quoting 5/79) may be obtained from the Registrar, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, with whom applications must be lodged by February 28, 1979 9395(A)

UNIVERSITY OF ST ANDREWS
DEPARTMENT OF ANATOMY AND EXPERIMENTAL PATHOLOGY

Applications are invited for the post of Technician (Grade 4) in the above Department The successful applicant will take charge of the dissecting room and embalming suite, participate in the preparation of material for display in the departmental museum and assist with the preparation of teaching material

Salary at appropriate point on scale £2,955 to £3,402 per annum (under review) Applications, with the names of two referees, should be sent to The Establishments Officer, The University, College Gate, St Andrews, Fife by February 8, 1979 9427(A)

CSIRO AUSTRALIA
Research Scientist
Division of Animal Health
Glebe, NSW

CSIRO has a broad charter for research into primary and secondary industry areas The Organization has approximately 7,000 employees—2,300 of whom are research and professional scientists—located in Divisions and Sections throughout Australia

Field: Immunochemistry/Microbiological Chemistry

General: The Division has a major programme on infection and immunity with a substantial interest in immunity at mucosal surfaces in relation to helminth parasites and microbial agents An immuno-chemist/microbiological chemist is now sought to work at the McMaster Laboratory in the above programme

Duties: To carry out research into aspects of immunity to gastro-enteric infections, especially collibacillosis Attention could be directed initially to fractionation of *E coli* antigens and assaying for immunogenicity The appointee would be encouraged to collaborate with others in the programme

Qualifications: A Ph D degree in a relevant discipline, or equivalent qualifications, supported by satisfactory evidence of research ability and experience appropriate to the field of study

Salary: Research Scientist or Senior Research Scientist · $A15,422 to $A22,405 p a. An outstanding applicant may be considered for appointment at a higher level

Tenure. Indefinite with Superannuation

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 201/566, should reach —

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 28th FEBRUARY, 1979

Applications in U S A and Canada should be sent to.—
The Counsellor Scientific, Embassy of Australia,
1601 Massachusetts Avenue, N W, Washington, D C, 20036,
U S A 9441(A)

Tropical Products Institute
Culham, Oxon

Food Technologist

. . . to join the Non-Cereals Section of the Institute working on processing problems for a wide range of fruit and vegetables In addition the food technologist will take an active part in the current work of the Section, which includes various projects in sugar and starch processing and the maintenance of formal and ad hoc heat preservation training programmes. The Section has extensive facilities including a transportable cannery used for training, varietal research and local preparation of bottled and canned samples for marketing trials in producing areas. There are opportunities to undertake overseas project work.

Candidates, normally aged under 30, must have a degree or equivalent in an appropriate discipline. Practical experience of commercial food processing is highly desirable. *Final Year Students may apply.* Appointment as Higher Scientific Officer (£4100–£5450) or Scientific Officer (£2840–£4410) according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 23 February 1979) write to Miss C A Hall, Tropical Products Institute, 127 Clerkenwell Road, London, EC1R 5DB. *Please quote ref SA/96/JD.*

9422(A)

Science
Group
CIVIL SERVICE

THE SOUTH STAFFORDSHIRE WATERWORKS COMPANY

SENIOR SCIENTIST

Salary range: £5,133 to £6,084 including supplement

A senior scientist, male or female, is required to work at the Company's central laboratory, to be relocated from Lichfield to Walsall during 1979

Duties cover all aspects of scientific control of water quality assurance and include development of new analytical methods

A good honours degree in chemistry is the minimum qualification required. Industrial analytical experience is desirable, though not essential.

Please write by March 1 to The Chief Engineer, The South Staffordshire Waterworks Co, 50 Sheepcote Street, Birmingham B16 8AR

9397(A)

MONASH UNIVERSITY
DEPARTMENT OF
PATHOLOGY AND
IMMUNOLOGY
JUNIOR RESEARCH FELLOW

Junior Research Fellow with biophysics qualifications or relevant experience, to help operate a Becton-Dickinson FACS—11 for fluorescence cell sorting in immunological and cancer research directed by Professor R. C Nairn Must be interested in flow cytometry and computer data analysis Position available immediately Salary $A13,606 to $A15,190 per annum Travel and removal allowance Temporary housing assistance Applicants should quote reference No 30865 Applications in duplicate to the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria 3168, Australia, giving age, qualifications, experience and names and addresses of three referees

9432(A)

THE KENNEDY INSTITUTE OF RHEUMATOLOGY

A postdoctoral or postgraduate
RESEARCH ASSISTANT

is required in the Division of Biochemistry to be involved in projects using culture techniques to investigate the cellular mechanisms involved in the control, development and repair of connective tissue Candidates should have a keen aptitude for research and previous experience of cell culture techniques The appointment is funded by the Medical Research Council until November 1980 Salary £3,500 to £4,500 depending on age and qualifications

Applications in writing giving full particulars of experience and qualifications and two referees to The General Secretary, The Mathilda and Terence Kennedy Institute of Rheumatology, Bute Gardens, Hammersmith W6 7DW.

9436(A)

GUY'S HOSPITAL MEDICAL SCHOOL
(University of London)
POSTDOCTORAL RESEARCH ASSISTANT

required to join a research group under Dr L Mallucci in the Department of Microbiology investigating the role of cell surface membrane components in cell growth regulation and cell differentiation Position available to December 1979 with the possibility of further renewal of grant

Salary in range £3,910 to £5,506 plus £450 London Allowance and superannuation.

For further details telephone Dr Mallucci

Applications to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT.

9055(A)

FELLOWSHIPS

THE ROYAL SOCIETY
THE BRITISH PETROLEUM COMPANY LIMITED
SENIOR RESEARCH FELLOWSHIP

Applications are invited by the Council of the Royal Society for The Royal Society — British Petroleum Company Limited Senior Research Fellowship, established by means of a five-year grant from the British Petroleum Company Limited for research in a subject field associated with the exploration, production and transport of fossil fuels and/or with the physical, chemical and mechanical properties of materials used in these operations The Fellow will be expected to have an interest in the application of his research to practical problems The appointment will be tenable in any appropriate university department or research Institution in the United Kingdom approved by the Council of the Royal Society Candidates, who must be citizens of the United Kingdom or the Commonwealth, must supply the usual personal details and give two referees Testimonials will not be considered The subject of the proposed research, and where it would be done, together with the name of the head of the department, whose consent should first be obtained, must be stated

The appointment, which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from October 1, 1979 (or another date to be arranged) Applicants should preferably be under 45 on October 1, 1979 The stipend scale will be £8,031 by £252 to £8,787 by £249 to £9,036 per annum, and the point of entry will be determined by Council Superannuation benefits will be provided Some provision for research expenses will be available

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and returned by March 31, 1979

9364(E)

POSTDOCTORAL FELLOWSHIP

Available from April 1, 1979 for a Ph D Graduate in biochemistry, virology, or related area interested in studying the structure and replication of mammalian and avian R N A viruses Applicants must have received the Ph D degree no earlier than April 1, 1978 Salary is $12,000 for the first year and $13,350 for the second.

Send curriculum vitae and references to Dr D G Scraba, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

The University of Alberta is an equal opportunity employer 9378(E)

THE ROYAL SOCIETY
BRITISH GAS SENIOR RESEARCH FELLOWSHIP

Applications are invited by th Council of the Royal Society for Tl Royal Society—British Gas Senk Research Fellowship for origin scientific or technological research a field of mathematics, chemistr physics or engineering (includu metallurgy and materials science, flu mechanics, thermodynamics, combustion, heat transfer, biochemistry, at computer science) related to the pr duction, transmission and use natural gas and its substitutes.

Candidates, who must be citizens the United Kingdom or the Commo wealth, should supply the usual pe sonal details and two referee Testimonials will not be considere Candidates should state the subject the proposed research and where will be undertaken It is expected th the successful candidate will spend significant proportion of his or h time in direct collaboration with I dustry; evidence of existing contac would be helpful Applicants shou obtain and give evidence of conse from the head of department or oth authority in the university and/or i dustrial establishments in which th research will be done.

The appointment which will be su ject to the Society's general regulatio governing research appointments, w be tenable for five years from Octob 1, 1979 (or another date to b arranged), and may exceptionally b renewable for a further two year such renewal being considered at th end of the fourth year of tenur Applicants should preferably be und 45 on October 1, 1979 The prese stipend scale is £8,031 by £252 £8,787 by £249 to £9,036 per annur and the point of entry will be dete mined by Council Superannuatic benefits will be provided Some pr vision for research expenses will b available

Applications should be made forms to be obtained from the Exec tive Secretary, The Royal Society, Carlton House Terrace, London SW1 5AG, and should be received n later than March 31, 1979 9365(E)

THE ROYAL SOCIETY
SENIOR RESEARCH FELLOWSHIPS

Applications are invited by tl Council of the Royal Society for to three Royal Society Senior Resear Fellowships for research in any scie tific subject, established by funds fro the Parliamentary Grant-in-aid. Th appointments will be tenable in a appropriate university department research institution in the Unit Kingdom approved by the Council the Royal Society Candidates, wh must be citizens of the United Kin dom or the Commonwealth, mu supply the usual personal details a give two referees Testimonials w not be considered The subject of tl proposed research, and where it wou be done, together with the name the head of the department, who consent should first be obtained, mu be stated

The appointments, which will b subject to the Society's general reg lations governing research appoir ments, will be tenable for five yea from October 1, 1979 (or another da to be arranged) and may be renewat for a further two years Applicar should preferably be under 45 October 1, 1979 The present stipe scale is £8,031 by £252 to £8,787 £249 to £9,036 per annum, and t' point of entry will be determined l Council Superannuation benefits w be provided Some provision for r search expenses will be available

Applications should be made forms to be obtained from the Exec tive Secretary, The Royal Society, Carlton Terrace, London SW1Y 5A and returned by March 31, 1979

9363(E)

FELLOWSHIPS continued

RESEARCH FELLOWSHIPS

Two postdoctoral Fellowships available for three years in the I C.R.F. Medical Oncology Unit, St Bartholomew's Hospital Salary range £4,882 to £6,080 plus £450 L.W.

1) A cytogeneticist is required to identify chromosomal abnormalities in non-Hodgkin's lymphoma and leukaemia, and to relate these abnormalities to the class of tumour defined by pathological and immunological criteria. Experience of chromosome banding techniques is essential, and a background knowledge of immunology or cytochemistry desirable.

2) An immunologist is required to study the distribution of malignant lymphoma cells and their precursors in lymph node and bone marrow biopsies of patients with lymphoma. Experience in detection of surface antigens, preparation and purification of antisera, or surface phenotyping techniques is considered essential.

Applications with curriculum vitae and two referees to Staff Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2. Closing date March 19, 1979. 9394(E)

RESEARCH FELLOWSHIP

Imperial Cancer Research Fund Laboratories,
Mill Hill, NW7

A postdoctoral Fellow is required to join a group working on the genetic and biochemical analysis of certain heat activated loci in Drosophila melanogaster, using cloned DNA fragments to investigate their organisation and expression. Experience in molecular biology desirable.

Appointment will be for three years. Salary with entry according to qualifications and experience within range £5,332 to £6,530.

Further information from Dr Ish-Horowicz (01-959 3236). Applications with curriculum vitae and names of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields WC2A 3PX by February 24, 1979

9428(E)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF PHYSICS

ESEARCH FELLOWSHIP IA

Applications are invited for a post-ctoral Research Fellow to join the edical Applications group in the partment of Physics, to work on e measurement of fast-neutron ectra in tissue with a view to timising conditions for fast-neutron erapy Previous experience with clear instrumentation, neutron ectroscopy, and/or computing uld be advantageous This post is pported by the M R C

The post is tenable for three years om March 1, 1979 Starting salary ll be on the appropriate point of e Research Fellow Grade IA scale, ,883 to £6,555, plus superannuation arting salary will not exceed £4,882

Further particulars are available om the Assistant Registrar, Science d Engineering, University of Bir-gham, P O. Box 363, Birmingham 5 2TT, to whom applications (three ies) including curriculum vitae l naming three referees should be t by Monday, February 19, 1979 lease quote ref ND2. 9371(E)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF PHYSICS

RESEARCH FELLOWSHIPS IN CRYOGENICS

Applications are invited for postdoctoral fellowships to work on the following projects

(1) Heat transfer in the reboiler/condensers of cryogenic plant, using model rigs and a full length test section
(2) Complex convective flow in stratified cryogenic vapours and liquids using Laser Doppler Anemometry
(3) Heat transfer and fluid behaviour of helium at 3,000 r p.m. for the development of large superconducting machines

Applications should have experience in mechanical/chemical engineering or low temperature physics

Salary in range up to £5.367 plus U S S benefits

Applications with names of two referees should be sent to Mrs E C P Sears, Staffing Section, The University, Highfield, Southampton SO9 5NH Please quote reference no 1607/R/Na. 9290(E)

LANCHESTER POLYTECHNIC

Coventry

Department of Biological Sciences

Postdoctoral Research Fellowship

(Tenable for up to 3 years from March 1, 1979)

£4,101 - £6,568

THE JOB The project is funded jointly by the SRC and ICI and involves the development of a method for the *in vitro* culture of perifused hepatocytes supported in a 3-dimensional matrix and its application to toxicological studies

REQUIRED Experience in animal cell culture and biochemistry Knowledge of cytological procedures would be an advantage

For further information applicants may contact Dr Sturdee or Dr Crisp by telephoning Coventry (0203) 24166

Faculty of Applied Science

Research Assistant in Cardiac Pacing

(Tenable for 1 year from March 1, 1979)

£3,192 - £3,330

THE JOB A statistical survey of patients with artificial cardiac pacemakers is required to attempt to identify those factors which cause a rise of stimulation threshold in certain patients The required collection of data will involve close collaboration with cardiac pacing centres in a number of hospitals

REQUIRED Applicants should have either an appropriate Honours Degree, or an appropriate HNC plus relevant experience

For further information applicants may contact Dr. Economides or Dr Duckers by telephoning Coventry (0203) 24166

For application forms and further particulars for the above posts please apply in writing enclosing a foolscap stamped and addressed envelope to the Personnel Officer, Lanchester Polytechnic, Priory Street, Coventry CV1 5FB, applications returnable by February 19, 1979 9404(E)

LINCOLN COLLEGE

(University College of Agriculture)
New Zealand

POSTDOCTORAL FELLOWSHIP

The Council of Lincoln College invites applications for a Post-Doctoral Fellowship tenable in the fields of Agricultural Microbiology (Ethanol Fermentation and/or Symbiotic Nitrogen Fixation), Entomology (Pest Management), or Environmental Sciences (Regional Development)

The object of the award is to contribute to the research programme at Lincoln College In addition to carrying out research, the successful applicant will be expected to participate in limited teaching activities without further emolument

The Fellowship will have a total value of NZ$10,000, part of which will be available for fares Tenure will be for one year in the first instance, but may be extended at the discretion of Council.

Conditions of appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the College

Applications for this Fellowship close on April 30, 1979. 9421(E)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from graduates in Chemistry or Physics to work with Dr J. L Duncan and Dr D C McKean on Infra-red Spectroscopy The successful applicant will undertake research on a variety of topics using a computer controlled, high resolution Fourier transform spectrometer Previous interactive experience, and an ability to develop software and undertake electronic maintenance is desirable

The post is funded by the Science Research Council and is available from April 1979 Appointment will be for one year in the first instance, with possible extension up to a maximum of three years

Commencing salary within Range 1A, £3,883 to £4,382 per annum, with appropriate placing

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by February 23, 1979

9375(E)

POSTDOCTORAL FELLOWSHIP

National Institutes of Health, U S A

To start any time between August and November 1979 Individual trained in cell biology, genetics, and/or biochemistry, to work in laboratory of L Leive on eukaryote membranes Fellowship for non-U S citizen, newly or up to four years postdoctoral Contact Dr Leive at NIH, 4/116, Bethesda, Md 20014, USA 9457(E)

FELLOWSHIPS—continued

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY

SPRING 1979 AWARDS

Next deadline: February 20, 1979

The long term fellowships will usually be for a period of up to one year but applications for a renewal for a second year are considered. To be eligible a candidate must hold a doctor's degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel EMBO long term fellowships are not, however, awarded for exchanges between laboratories within any one country. Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel.

The deadline for applications for the 60 fellowships to be awarded in April 1979 is February 20, 1979. Because the selection procedure involves an interview this deadline should be respected.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R. Germany.

9278(E)

STUDENTSHIPS

AGRICULTURAL RESEARCH COUNCIL STUDENTSHIP

Applications are invited for a Studentship to work in the Protein Section of the Meat Research Institute.

The project is to investigate the turnover and remodelling of the connective tissues in relation to growth and development.

This will consist of radioactive incorporation studies together with autoradiography, the chemical analysis of connective tissue components and an examination of the cellular mechanisms for resorption of tissue.

The project is expected to form the basis for a Ph.D thesis in the University of Bristol and the appointment will commence in October, 1979.

Candidates should possess a First or Upper Second Class degree in Biochemistry

Further particulars of the award and allowances together with Application Forms may be obtained from the Personnel Officer, Agricultural Research Council, Meat Research Institute, Langford, Bristol BS18 7DY

9431(F)

NATIONAL VEGETABLE RESEARCH STATION A R C RESEARCH STUDENTSHIP Applications are invited for an Agricultural Research Council postgraduate Studentship for research on the mode of action of systemic fungicides active against *Bremia lactucae* (lettuce downy mildew) and variation for sensitivity in the fungus to these chemicals The work will involve laboratory, glasshouse and field studies The studentship will be tenable from October 1, 1979, for up to three years at the NVRS with a view to reading for the degree of Ph D at Birmingham University Candidates should hold or expect to obtain in 1979 a first or upper second class Honours degree in Plant Science with particular emphasis on at least one of the following disciplines. plant pathology, plant biochemistry, plant protection and genetics Further particulars and an application form (to be returned by February 20, 1979) from the Secretary, National Vegetable Research Station, Wellesbourne, Warwick CV35 9EF 9450(F)

Strathclyde Regional Council—Department of Education

GLASGOW COLLEGE OF TECHNOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES

S.R.C. Studentship in Biochemistry or related subject areas to work on the "sodium pump" in essential hypertension A first or upper second class honours degree is a prerequisite for the award. The post has to be filled by April 30, 1979. Write or phone for further particulars to Dr T. Duffy, Department of Biological Sciences, Glasgow College of Technology, Cowcaddens Road, Glasgow G4 0BA. Telephone 041-332 7090, Ext. 283 or 340 not later than February 12, 1979.

E. MILLER Director of Education

9425(F)

GRASSLAND RESEARCH INSTITUTE

Hurley, Maidenhead, Berkshire

POSTGRADUATE STUDENTSHIP

Applications are invited for the award of a postgraduate studentship funded by the Agricultural Research Council and tenable at the Grassland Research Institute for a period of three years commencing on October 1, 1979.

The Grassland Research Institute is an Associated Institutio of the University of Reading, where the successful applican will be registered for a higher degree.

There is a choice of projects and candidates should indicate clearly for which project they are applying.

Project 1 To identify the important factors influencing the economic assessment of dairy systems, including the effects of climate, soil and management. The study will involve the construction of a model to determine the response of the system to these factors with a view to improving output Candidates should have or expect to obtain a first or upper second class honours degree in a quantitative subject and have an interest in agriculture.

Project 2 To investigate the effects of early season management and water availability on the nitrogen uptake an seasonal pattern of grass growth The investigation will b field based and will include studies of tillering, herbage growth, nitrogen uptake and soil water.

Candidates should have or expect to obtain a first or uppe second class honours degree in Agricultural Botany or Plan Science.

The studentship comprises a tax-free maintenance allowanc of £1,610 per annum plus compulsory fees and travellin expenses.

Applications by February 28, 1979, to the Secretary, fro whom further details may be obtained.

9444(F)

A.R.C. ANIMAL BREEDING RESEARCH ORGANISATION

DEPARTMENT OF APPLIED GENETICS

POSTGRADUATE RESEARCH STUDENTSHIP

Applications are invited for an A R C Research Studentship tenable i Edinburgh for three years from October 1979

The project deals with the evaluation of foreign pig breeds, involvin statistical analysis of experimental data and theoretical study of methods The successful applicant will be expected to register for a higher degree and there may be an opportunity for additional coursework in Anima Breeding.

Candidates should possess, or expect to obtain, a good honours degre in agricultural science, biology or statistics, and have an interest i biometrical work

Awards include a tax-free allowance, payment of fees and other ex penses Full details and application forms are available from the Secre tary, A R C Animal Breeding Research Organisation, West Mai Road, Edinburgh EH9 3JQ, Scotland Applications should be made b March 3, 1979

9439(F)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE FOR RESEARCH ON ANIMAL DISEASES

POSTGRADUATE STUDENTSHIP

Applications are invited from suitably qualified candidates for a Studentship, funded by Standard Telecommunications Laboratories in conjunction with Hoechst Ltd., for work leading to a higher degree

The objective of the research will be to develop new methods for prevention of diseases due to trace element deficiencies in cattle and sheep and this will involve measuring the absorption, retention and excretion of the copper and selenium in a number of compounds currently used for the control of deficiency states and investigating the advantages and limitations of using the newly-developed controlled-release glasses

Candidates should possess a First or Upper Second Class degree in a biological science or expect to obtain this qualification at the end of the academic year.

Application forms from Secretary, Agricultural Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berks., quoting reference 304

9382(F)

GRANTS & SCHOLARSH

POSTGRADUATE RESEARCH

DEPARTMENT OF BIOLOG

PRINCETON UNIVERSI

N I H postdoctoral training positions for recent Ph D's in areas of biochemical and ge aspects of cell differentiation (D Cline, E Cox, A Newton, P Sc cell biology and development (D Bonner, E Cohen, M Steinberg Witman), and behavioural and lation genetics (Drs J Endler Gould, R May, W Quinn) are able beginning summer, 1979 A cations should include curric vitae and summary of research terest

Closing date April 2, 1979 quiries or applications should be to Dr E C Cox, Chairman, D ment of Biology, Princeton Unive Princeton, N.J 08540

Princeton University is an Opportunity/Affirmative Action ployer.

938

THE NATIONAL KIDNEY SEARCH FUND invites applic for grants for renal research a within the United Kingdom Ap tion forms are available from Secretary, 184B Station Road, Ha Middlesex HA1 2RH (01-863 44

9408

GORDON RESEARCH CONFERENCES

"Frontiers of Science"

1979 SCHEDULE - NEW HAMPSHIRE

	Colby-Sawyer College New London, NH	**New Hampton School New Hampton, NH**	**Kimball Union Academy Meriden, NH**	**Tilton School Tilton, NH**
June 11-15	*Elastin	Nucleic Acids	Cyclic Nucleotides	Theoretical Biology and Biomathematics
June 18-22	Nuclear Chemistry	Environmental Sciences: Air	Lipid Metabolism	Animal Cells and Viruses
June 25-29	Catalysis	Proteins	Atherosclerosis	Carbohydrates, Chemistry of
July 2-6	Fiber Science	Bacterial Cell Surfaces	Interfaces, Chemistry at	Polymer Colloids
July 9-13	Polymers	Heterocyclic Compounds, Chemistry of	Bones and Teeth, Chemistry, Physiology and Structure of	Nuclear Structure Physics
July 16-20	Elastomers	Organic Reactions and Processes	Enzymes, Coenzymes and Metabolic Pathways	Biomaterials, Science and Technology of
July 23-27	Corrosion	Natural Products	Point and Line Defects in Semiconductors	*Food Microbiology
July 30-August 3	Food and Nutrition	Statistics in Chemistry and Chemical Engineering	Toxicology and Safety Evaluations	Muscle, Ionic Channels, and Other Excitable Membranes
August 6-10	Medicinal Chemistry	Inorganic Chemistry	Glycoproteins and Glycolipids	*Epithelial Differentiation and Keratinization
August 13-17	Separation and Purification	Analytical Chemistry	Fluids in Permeable Media	Laser Interaction with Matter
August 20-24	Cancer	Adhesion, Science of	Ion Exchange	Transport Phenomena in Lipid Bilayer and Biological Membranes

	Proctor Academy Andover, NH	**Holderness School Plymouth, NH**	**Brewster Academy Wolfeboro, NH**	**Plymouth State College Plymouth, NH**
June 11-15	Free Radical Reactions	Physical Organic Chemistry (Hydrocarbon Chemistry)	Space Plasma Physics	*Red Cells
June 18-22	Plant Cell and Tissue Culture	Mammary Gland Biology	Magnetic Resonance	Calcium Phosphates
June 25-29	Polyamines	Biological Regulatory Mechanisms	Atomic Physics	Molecular Pharmacology
July 2-6	Cell Contact and Movement	*Analytical Pyrolysis	Nonlinear Optics & Lasars	Fuel Science
July 9-13	Physical Metallurgy	Solids, Chemistry and Physics of	Molecular Pathology	Quantum Solids and Fluids, Dynamics of
July 16-20	Energy Coupling Mechanisms	Chemotherapy of Experimental and Clinical Cancer	Macromolecules and Behavior	Quantitative Structure Analysis
July 23-27	Organic Photochemistry	Drug Metabolism	Microbiological Degradation	Tumor Immunology
July 30-August 3	Developmental Biology	Fertilization and the Activation of Development	Ceramics, Solid State Studies in	*Ion Containing Polymers
August 6-10	Coatings and Films, Chemistry and Physics of	Postharvest Physiology	Micellar and Macromolecular Catalysis	Magnesium in Biochemical Processes and Medicine
August 13-17	Catecholamines	Liquids, Chemistry and Physics of	Few Body Problems in Chemistry and Physics	*Dynamics of Gas-Surface Interactions
August 20-24	Elementary Particle Interactions	Inorganic Geochemistry	Molten Salts and Metals	*Remote Sensing of the Earth's Surface from Space

*NEW CONFERENCES—1979

Analytical Pyrolysis
Dynamics of Gas-Surface Interactions
Elastin
Epithelial Differentiation and Keratinization
Food Microbiology
Iron Containing Polymers
Red Cells
Remote Sensing of the Earth's Surface from Space

CALIFORNIA

Miramar Hotel, Santa Barbara

June 25-29 Marine Natural Products

Programme. The complete programme for the 1979 Gordon Research Conferences will be published in *Science*, March 9, 1979. Reprints will be available on request.

Requests for applications to the Conferences, or for additional information, should be sent to:

Dr Alexander M. Cruickshank, Director
Gordon Research Conferences
Pastore Chemical Laboratory
University of Rhode Island
Kingston, RI 02881, USA
Tel: 401-783-4011/3372

FIXED CONFERENCE FEES—1979

New Hampshire

*Resident Fee: $185 includes $50 registration fee, room and meals.
*Non-resident Fee: $150 includes $50 registration fee and meals.
Guest: $135 for room, meals.
Deposit: $30 is required for all participants and guests.

California

*Resident Fee: $200 includes $50 registration fee, room and meals.
Guest: $150 for room, meals.
Deposit: $30 is required of all participants and guests.

*Fixed fees cannot be prorated or reduced.

9423(C)

SYMPOSIA

THIS TENTH ANNUAL INTERNATIONAL SYMPOSIUM

will deal with the control of national food quality, chemical, metabolic and microbiological aspects, health foods, additives and contaminants, healthy eating, special processing and packaging aspects, food and the brain and infant nutrition. Speakers will include experts from the U.K., U.S.A., Holland, Germany, Italy, Switzerland and Poland. The keynote lecture, "Food and Health from Conception to Extreme Old Age", will be given by Dr Elsie M. Widdowson.

Programmes containing application forms are available from:

The Secretary
National College of Food Technology
(University of Reading)
St. George's Avenue
Weybridge, Surrey 9453(M)

COURSES

INSTITUTES OF PSYCHIATRY, NEUROLOGY AND OPHTHALMOLOGY

(British Postgraduate Medical Federation,
University of London)

POSTGRADUATE COURSE IN NEUROCHEMISTRY

This is a ONE-YEAR Course leading to the degree of M.Sc. in Neurochemistry. It offers graduates in science and medicine training in basic and applied neurochemistry. The Course consists of lectures, seminars and laboratory work and, in the second and third terms, research on a neurochemical topic.

Applicants should have an honours degree in Biochemistry or a Biological subject with some Biochemical component, or be Medically qualified. The Course is recognised by the Medical Research Council as an Advanced Course for the award of Training Scholarships to suitably qualified applicants.

Application for the Academic year October 1979 to July 1980 should be addressed to the Secretary, Course in Neurochemistry, Department of Biochemistry, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, England. 9443(D)

BRITISH COUNCIL COURSE

THE LYMPHOCYTE

2–14 December 1979

The aim of this course is to familiarise participants with the latest advances in Britain in the immunology of the lymphocyte and to update their knowledge of current concepts of the role of the lymphocyte in adaptive immunological processes. Lectures, discussion periods, demonstrations and some practical work will highlight central developments such as the role of the major histocompatibility complex and recent technological advances.

The Directors of Studies will be Professor I. M. Roitt and Dr P. M. Lydyard of the Middlesex Hospital Medical School, London, where the course will be based.

Members of this course will be postgraduates, mainly working outside Britain, who are laboratory investigators or clinicians with 2–3 years experience in immunology or allied fields.

Fee: including demi pension, £475.

Application forms, which should be in London by July 1, 1979, are available from your local British Council Representative or from The Director, Courses Department, The British Council, 65 Davies Street, London W1Y 2AA, Great Britain. 9458(D)

EMBO COURSE ULTRACRYOMICROTOMY

An advanced practical course on ultracryomicrotomy will be held at EMBL Heidelberg from May 2-4, 1979. Instructors will include Dr K. Tokuyasu, UCLA San Diego. Participants will be limited to a maximum of 10 and preference will be given to those having some experience with the technique. Limited funds will be available to cover travel expenses in some cases. Applicants should write before March 1, giving details of relevant interests to Dr G. Griffiths, EMBL, Postfach 10.2209, 69 Heidelberg, Fed. Rep. Germany.

9430(D)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF METALLURGY AND MATERIALS SCIENCE

ONE YEAR M.PHIL. COURSE IN MATERIALS TECHNOLOGY

The University has introduced a new degree, the Master of Philosophy in Materials Technology. The course aims to give training in the Materials research techniques and technology most relevant to the needs of development laboratories and industrial concerns.

Each student will undergo training in collaboration with a particular industrial firm. The lecture programme will be tailored to individual needs and the student will undertake a research project chosen for its direct relevance to the collaborating concern. Applications are invited from persons who possess or expect to get a good honours degree in subjects such as Metallurgy, Materials Science, Engineering Physics and Chemistry.

The course has been accepted as qualifying for the award of S.R.C. Advanced course Studentship. Applications and enquiries should be sent to the Course Convenor, Dr B. Ralph, Department of Metallurgy and Materials Science, University of Cambridge, Pembroke Street, Cambridge. 9380(D)

ELECTRICAL DISCHARGES IN IGNITION AND COMBUSTION

A Post Experience Course will be held at Imperial College from May 1 to 3, 1979. Details from Dr M. D. Carabine, Chem. Eng. and Chem. Tech., Imperial College, London SW7 2BY. Tel: 01-589 5111, Ext. 1906.

9434(D)

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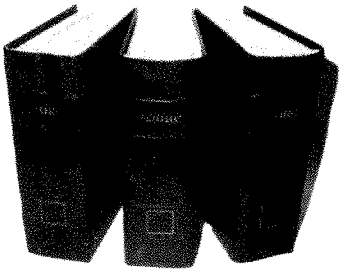

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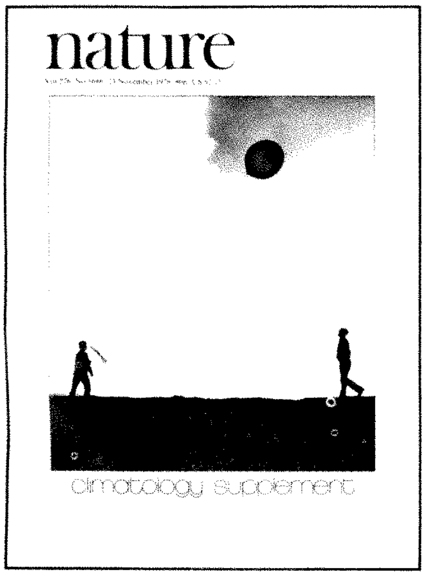

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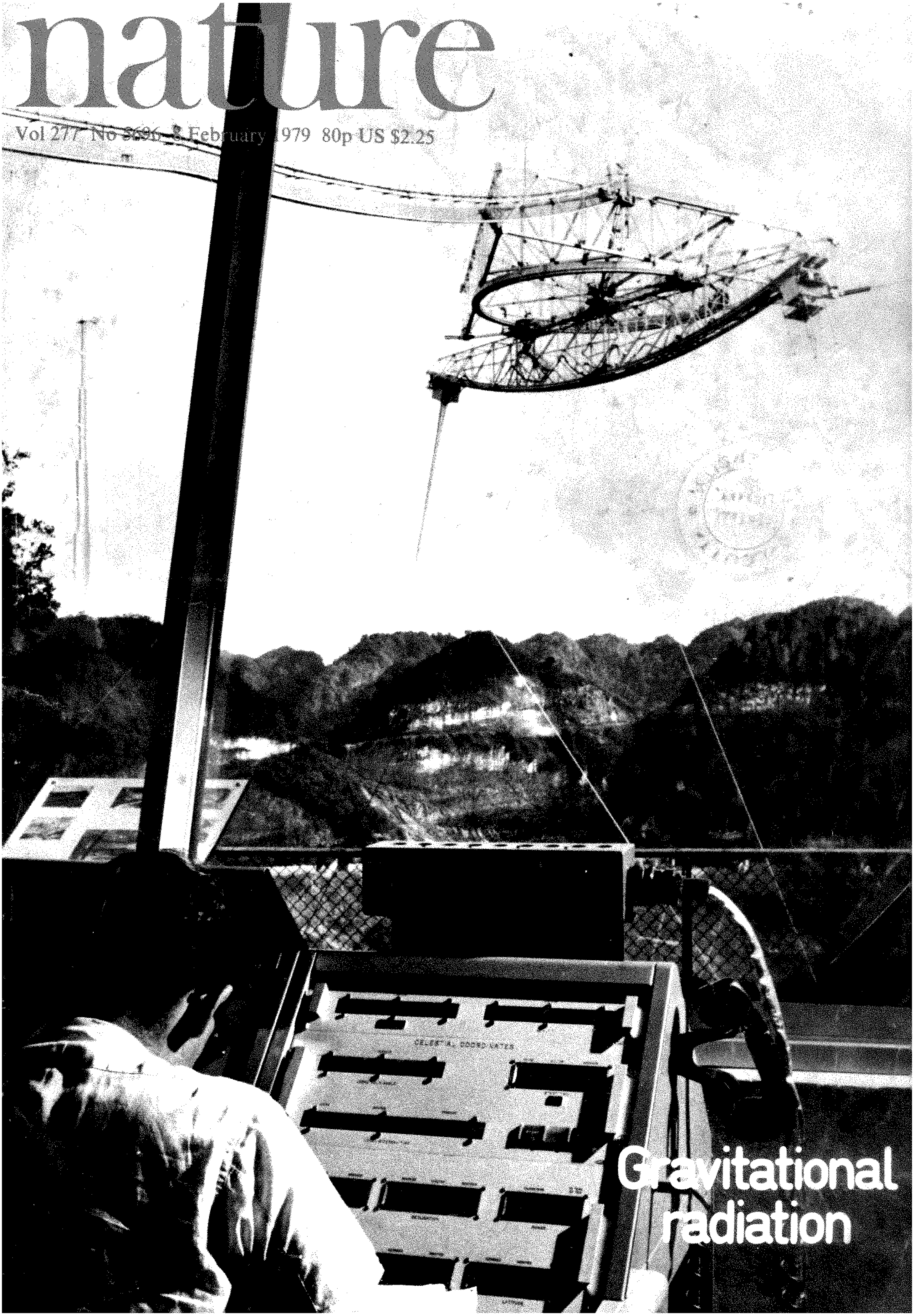

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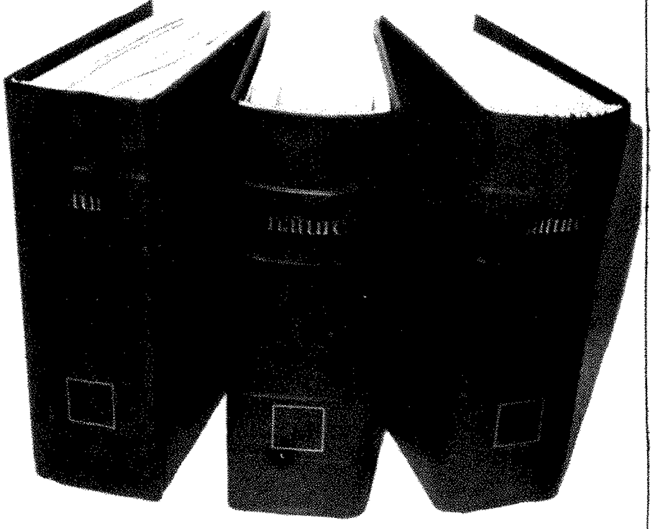

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Cover picture

The discovery of gravitational wave effects in a binary pulsar system using the Arecibo radio telescope is reported on page 437.

[Photo: Curt Gunther/Camera Press]

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- Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.
- 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système International. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; exp (a) is preferred to e^a if 'a' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note:

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In the article 'measurements of general relativistic effects in the binary pulsar PSR1913 + 16' by J. H. Taylor *et al.* on page 437, Fig. 3 is shown incorrectly above the legend to Fig 2 and vice versa.

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8 February 1979

Populism . . . elitism . . . or a middle way?

THE newly formed Science, Technology and Society Association started life with a suitably large splash last week in London by having Mr Tony Benn, UK Secretary of State for Energy and the association's president, talk on the democratic control of science and technology. The association has emerged from the SISCON (Science in a Social Context) project which, over the past few years, has brought together various groups in higher education in Britain interested in the broader issues of science. The plan now is to reach beyond the academic world and not just into other areas of education. Its first newsletter speaks of hoping to draw members from industrial managers, trade unionists, politicians, members of the public "to confront scientists with the implications of their researches", teachers and journalists. An unfortunate omission is mention of those very scientists and technologists whose work raises so many issues. And at the first meeting there was also a sad lack of those same scientists and technologists.

Mr Benn's not entirely unpredictable line was that the questions raised by developments in science and technology were too important to be the subject of closed discussion amongst an elite. He drew two examples, from the military world and the field of nuclear power. The disciplined hierarchy of the military, comparable with monastic orders, could have all sorts of influences of which the public were totally unaware; enormous expenditures in what was now a highly technological defence force were essentially secret. Again, nuclear power, regardless of the merits of those who have devoted their careers to it, raises the some problems of democratic control and tightfistedness over the flow of information. Mr Benn plans to postpone a decision on the commercial fast-breeder reactor for as long as possible, not because of doubts on the technological side but because, he claims, he is as yet not satisfied that the democratic machinery exists to bring out all relevant information.

One of the prices of high technology and high living standards, he pointed out, is a greater vulnerability of society to industrial disputes, technological and natural disasters and war. And as a consequence civil liberties can be eroded—we might find ourselves backing into a police state by means of high technology, and abandoning our values. We cannot, declared Mr Benn, discuss technology in isolation from the much neglected subject of societal values. And if we do not safeguard these values with democratic institutions we are in danger of trading liberties for colour television sets.

Mr Benn, of course, has great faith in the ballot box, in parliament and in fully informed ministers (not as managers so much as members of the public elected as it were to the board of directors) If the public, through elections, can have a voice in the country's social and economic policy, why not also in policy for science and technology?

It is possible to go a long way with Mr Benn's thinking on accountability, and yet to find the ballot box too facile a symbol of that accountability. As he himself admits, some do not vote for the government they believe to be most suitable but (for example) for the candidate who is for or against fluoridation. In all the complexity of motives for voting, and in all the manoeuvring a government does to try and ensure that it is re-elected, what hope that a stable policy for science and technology be central to anyone's thoughts? Yet without stability and rational policies, scientists and technologists who, more than almost any other sector of the community, can move in international circles will simply get up and go where the climate is more settled. And the idea of complex developments with necessarily vast ranges of uncertainty becoming an area of populist concern is bound to strike terror in the hearts of those patiently and quietly trying to accumulate the relevant knowledge

But whilst we ought to be wary of a sort of free-ranging democracy which is alien to science itself, if the obverse is a return to monastic secrecy, that too must be resisted. Certainly scientists and technologists are a very different breed from politicians, and distrust is to be found on both sides of the fence. But somehow we as scientists have to raise our deplorably low political awareness just as we have to hope that more than a handful of politicians like Mr Benn will find they can move freely and happily within the world of science and technology. Here the Science, Technology and Society Association should have a major role to play. One of its objectives should be to incorporate into the educational system more courses which will make potential scientists and technologists think about public issues. At present it is too easy to become fully qualified without such a training If this were to make for wiser, more mature judgement by us, the practitioners, and if at the same time an increased number of politicians were able to appreciate the issues in more than terms of short term gain, then science and technology policy, founded on rational dialogue, would benefit immeasurably. And that, presumably, is what Mr Benn would wish ☐

Doctors claim A-bomb tests linked to rise in child leukaemia deaths

David Dickson reports on a statistical survey in Southern Utah

RESEARCH workers at the University of Utah claim to have discovered a two-and-a-half fold increase in the leukaemia death rate among children exposed to fall-out from atomic bomb tests carried out in the Nevada desert in the 1950s.

In one of the first systematic studies of civilians exposed to fall-out from the tests, the research workers have found a particularly large increase in the number of leukaemia deaths in children born around 1951—the beginning of the testing period—and that leukaemia death rates returned to their previous levels after the testing was stopped in the early 1960s.

The results of the study have already been passed to federal officials in Washington in a series of meetings last month, but have not yet been published. And although the research workers are cautious about interpreting a correlation as indicating a causal relationship the results of the study will provide a major boost to over 100 cancer patients and relatives of those who have died from cancer in areas surrounding the test site, who are currently suing the US government for a total of $232 million in damages.

" This is the bombshell that is going to establish beyond doubt that these claims are on solid ground," Mr Stewart Udalla, a former Secretary of the Interior, who is now in private law practice and is pressing the case against the government, said last week.

Residents of Southern Utah have for years suspected that an apparently abnormal level in the number of cancers in the area was linked to fall-out from the A-bomb tests. The two towns of Parowan and Paragonah, for example, with a combined population of 1,800, experienced four cases of leukaemia from 1956 to 1967, two to three times the expected rate.

So far, however, evidence has remained sketchy and largely anecdotal, with insufficient data to establish any significant correlation. Two weeks ago, for example, it was learned that a study carried out in 1965 by a scientist with the US Public Health Service's Division of Radiological Health, who claimed to have shown an excessive level of leukaemia among residents of two Utah counties exposed to fall-out, was ignored and subsequently forgotten.

Interest in a possible correlation between the tests and civilian deaths was revived last year, when evidence was presented to a congressional subcommittee that an excessive incidence of leukaemia had occurred among soldiers who took part in exercises while the tests were underway (a claim which has recently been substantiated by the Center for Disease Control).

Such evidence prompted the University of Utah's research team, headed by Dr Joseph L. Lyon, of the Department of Family and Community Medicine in the College of Medicine, to carry out a detailed study of child deaths in the state between 1944 and 1975. The results of their study are about to be published in *The New England Journal of Medicine.*

Dr Lyon and his colleagues focused their attention on children under 15 who died in 17 counties in the south of the state, which experienced relatively high fall-out from 26 tests between 1951 and 1958. Radioactive debris from the tests was carried eastwards by the wind from the Nevada test site into Utah.

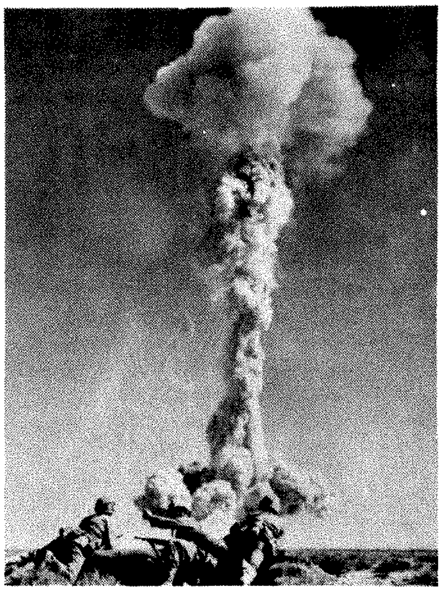
The children were divided into two groups for comparison: a "high exposure" group, who died 1951–58; and a "low exposure" group who died before 1951 or were born after 1958.

Analysis of the death certificates showed the leukaemia mortality rate among children in the low exposure group in these counties to be about half that in the remainder of the state, and indeed the rest of the US—a fact which is unexplained, but may be related to the ascetic life-style of the Mormon communities which predominate in the area.

In the high exposure group, however, although leukaemia mortality started off equally low, it climbed through the 1950s to reach a peak slightly higher than the US average in the early 1960s, subsequently falling back towards its previous level. This group did not reveal any excess for other childhood cancers.

The greatest excess in leukaemia deaths occurred in the 10–14 age group, where the number of deaths rose from between one and two per 100,000 to almost 10 per 100,000 around 1961—implying a particularly high incidence of leukaemia among those born towards the beginning of the testing period, with the disease causing death 10 to 14 years later.

The research workers found that there was a slightly lesser elevation in the 5–9 age group—those who were born around 1956—and little excess among the 0–4 age group. Furthermore the standard mortality ratio for the two groups for the whole age range was slightly higher for boys than for girls.



Nevada bomb test, 1952, watched by US marines

Even the children who lived in counties which had not experienced the high fall-out rates showed a slight increase in leukaemia mortality. But over half the excess deaths (16 out of 30) occurred in those counties which had been subject to the heaviest fall-out, even though these containd only 10% of the population.

Dr Lyon and his colleagues are being cautious about their results. They do not claim to have demonstrated that the excess leukaemia deaths were in fact due to the bomb tests, merely that there is a statistical correlation between the two. Their evidence is already being studied by other statisticians.

However, enough data has been produced by the study to convince others that when the Atomic Energy Commission told Utah residents in the early 1950s that the fall-out they were receiving was, to quote public statements, "far from hazardous" and presented "no danger" it was being grossly misleading—even admitting that the statements may have reflected the state of knowledge at the time.

Prompted by results such as those contained in the new study, President Carter has directed Mr Joseph Califano, Secretary of the Department of Health, Education and Welfare, to re-evaluate the findings of all earlier studies of the incidence of leukaemia in Utah, particularly in the south-west.

DHEW is expected to name the University of Utah researchers as the principal investigator in a major follow-up study. But whatever emerges, it may be too late to prevent what Mr Udall has called the "terrible tragedy" that took place 20 years ago. □

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Budget cuts cause upheavals in earthquake research

David Dickson describes how a sociologist's misbehaviour has led to a major gap in efforts to restrict the damage caused by earthquakes

TWO YEARS ago, Los Angeles City Council member Howard Jarvis—later to achieve fame as author of California's tax-cutting Proposition 13—led a successful campaign on behalf of the city's apartment owners to prevent signs being posted on private buildings liable to collapse during an earthquake. Some months later, sociologists found in a survey that over 90% of the inhabitants of the city, where more than 20,000 buildings are considered to be inadequately protected against earthquakes, thought that the posting of such signs would be a good idea.

Professor Ralph Turner of the University of California in Los Angeles, who heads the research team which conducted the survey as part of a wider project, claims that if the results had been known previously, the city council might have acted differently, and that they illustrate the type of role that the social sciences could play in mitigating the damage caused by earthquakes.

Yet, as the result of congressional actions last summer, National Science Foundation funds for social science research in this area have been savagely cut back. The foundation has been forced to reject all proposals for new projects so that existing ones can be completed. And even in the next fiscal year, funds for such policy research, according to the budget request now being submitted to Congress, will only be one-fifth of the amount originally requested for 1979.

Two years ago, the picture looked very different. With growing concern about a (supposedly) imminent earthquake in California, President Ford requested in his last budget proposal that spending on earthquake hazard reduction programmes at the NSF be increased from $12 million in 1977 to $32 million in 1979 (with an even larger increase at the US geological survey). Last year President Carter responded to legislation passed by Congress by setting out a national earthquakes hazards reduction programme. This stated that earthquakes posed "perhaps the greatest single-event natural hazards faced by the nation" (estimates put the property at risk at $2,300,000 million), and assigned the NSF to carry out basic and applied research on earthquake engineering and policy.

Then along came Senator William Proxmire. During hearings on the NSF budget, it emerged that a professor of sociology at the university of Colorado, Dr J. Eugene Haas, who had received NSF contracts for more than $500,000 to carry out research into the socioeconomic and political consequences of earthquake prediction, had used the money for, among other things, taking his girl friend on a trip to Honolulu, Tokyo and Hong Kong.

Congress is seldom kind to social scientists, and this misbehaviour, for which Professor Haas was subsequently dismissed from the university, was sufficient for the House Appropriations Committee to reduce the NSF's request for earthquake hazard research from $26.4 million to $17.4 million. Within this total, the amount to be allocated to social science policy research was reduced from $4.8 million to $800,000, the remainder being divided between siting and engineering design studies.

NSF officials were accused by the appropriations committee of mismanaging the funds awarded to Professor Haas. They were also stung by the claim that similar work was being carried out in other agencies, since they say these agencies turn to the foundation for their research support. Nevertheless the result has been that the NSF has had to reject all new proposals for research on the policy aspects of earthquake mitigation.

After an earthquake: devastation on the sidewalk of a southern California town.

These projects involved assessing the reaction on social and political institutions of information about earthquakes, as well as of the earthquakes themselves. "It is not just a question of needing more research. This is a subject that has never been adequately developed because there has never been a long-term commitment to provide support", Dr Robert Olsen, a social scientist of the Seismic Safety Commission in Sacramento, California, who has chaired a panel on the potential contribution of the social sciences, said last week.

"Many of our problems in reducing earthquake damage relate to legal and institutional considerations that affect the use of technical knowledge and its translation into public policy. The budget reductions mean that the lack of sufficient high quality people willing to undertake such projects is likely to remain."

Dr Olsen's brother, and associate professor in political science at the University of Redlands outside Los Angeles, is one of those who have proposed a major study to the NSF for which he has been told there is no money. The project would involve a two-year investigation in the San Bernardino area of how seismic safety issues are perceived by decision makers. "We shall be putting the proposal in again, and if it flies, it flies. Otherwise this is the last shot. Personally, the lack of money is driving me back into more traditional areas of social science."

Some feel that, despite the tough budget year, the NSF could be more aggressive in its approach to Congress. At present the foundation is requesting a level of funding for 1980 little higher than that to which it was reduced in the current year. "We will do what we can to get this raised, but it would have been easier if they had asked for a little more", the aide to one Californian congressman said last week.

Others feel it may take a major earthquake before research money starts flowing in. According to Dr Carl Kisslinger, president of the American Geophysical Union, the mayor of Pinotep Nacional in Mexico has claimed that public reaction to an earthquake forecast by US scientists did more damage to the economy of his town than the 7.5 magnitude shock that hit the area last August. ☐

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US scientists want 40% cut in defence spending

A GROUP of six scientists and military analysts has produced an "alternative military policy" for the United States which, it says, would buy a "better and safer defence" at only 60% of the cost of the present policy.

The Boston study group outlines its new strategy to reduce military spending in a 350-page book. *The Price of Defense,* which is being published shortly after President Carter has sent Congress a budget request for 1980 for a 3% real increase in defence spending.

The group says that the study is meant to "stimulate broad and serious debate of overall American military needs and foreign policy"—a debate which it claims has not taken place in the United States for many years.

Speaking at a press conference last week, Dr Martin Moore-Ede, professor of physiology at Harvard University, explained that the Boston group's proposed reduction of 40%—or $50 billion a year—in American defence expenditure would cut out "inertia, obsolescence and temptation: the inertia accounts for the entrenchment of high military budgets during peacetime, the obsolescence we cut are the easily-targeted, expensive and vulnerable remnants of the technological past, and the temptation we remove is the excess weaponry and forces that this nation possesses."

More specifically, the study recommends eliminating all of the United States' one thousand land-based intercontinental ballistic missiles over five years, although it concedes that a hundred of the latest Minuteman III ICBMs might be retained longer "as a hedge." All strategic nuclear bombers would also be retired, perhaps over eight years, on the grounds that they are "vulnerable, expensive and obsolete."

The Boston group argues that the retention of 31 Poseidon submarines, each carrying a maximum of 16 missiles, would leave the US with a virtually invulnerable nuclear deterrent sufficient to "wreck every Soviet and allied city and industrial district, plus many extra military targets". It sees no need for the new series of Trident submarines, the first two of which are under construction, and proposes cancelling them.

The Department of Defense's research and development activities would be severely pruned. The Pentagon would be left with only $100 million a year to spend on basic research: the group proposes to transfer the remaining $300 million to a new national technology foundation to "stimulate R & D in areas of expensive high technology where private industry is not yet willing to undertake the work". Applied research and development would be cut by two thirds.

The Boston group analysed the social effects of transferring $50 billion from the military to the civilian sector of the economy, and estimates that the net effect would be a gain of two million jobs "because defense dollars create far fewer jobs than monies spent in other sectors of the economy."

Clive Cookson

The Price of Defense. *Times Books, New York, at $15.00. Clive Cookson is with The Times Higher Education Supplement.*

UNCSTD meeting split on transfer of technology

DESPITE considerable opposition, the transfer of technology to developing countries will occupy a prominent, if controversial, position on the agenda of the United Nations Conference on Science and Technology for Development, in Vienna next August.

This emerged from the third meeting of UNCSTD's preparatory committee, which ended in New York on Monday, and at which delegates from a number of developed countries—in particular the United Kingdom, the US and West Germany—had argued that the issues involved were already being adequately debated in other forums.

In an address to the meeting, for example the British delegate, Dr Dennis Osborne said that attempts to duplicate negotiations on a code of conduct for the transfer of technology already underway elsewhere in the UN system "would lead to confusion and delay in reaching agreement".

Many of the recommendations in the UNCSTD secretariat's preliminary draft programme of action on this topic were "unacceptable to my government in their present form," Dr Osborne said. However Mr Frank Joao da Costa, Secretary General of the conference, told the preparatory committee that the proposed agenda for Vienna and the draft programme merely reflected the wishes expressed by member countries—and that since the transfer of technology was high among the concerns of many developing countries in their national and regional papers, it would have to be addressed by the conference.

In its closing plenary session on Monday morning, postponed from last Friday to allow additional time for discussion, the committee accepted a resolution submitted by the Tunisian delegate on behalf of the Group of 77 to restructure the draft programmes of action under three 'target areas': reinforcement of the scientific and technological capacities of developing countries; restructuring the conditions of access to scientific and technological knowledge as an integral part of the new international economic order; and better coordination of the scientific and technological activities undertaken within the UN system.

THIRD WORLD TECHNOLOGICAL DEVELOPMENT
UNCSTD COMMITTEE STILL UNDECIDED

"If they delay long enough, the technology they give us will be out of date"

Despite agreement on the formal changes which they wish to see in the programmes of action, however, the Group of 77, which had established a working party during the committee meeting, were unable to come to any agreement on the substantive issues which the preliminary draft of the programme has raised.

Members of the group, to which over 120 developing countries now belong, have therefore agreed to hold a further meeting before the next preparatory committee meeting, which takes place in May, and to create a mechanism for continuing discussion of the issues up to UNCSTD itself.

According to some observers, it was this strategy on the part of the developing countries which led to the relative success of last year's UN conference on technological cooperation between developing countries. "If this is the case, then the game will have been worth a candle" one of them said.

Delegates at the meeting were unable to agree on whether there should be a fifth meeting of the preparatory committee before UNCSTD, although if such a meeting is called 26 June–6 July has been put aside for it in New York.

David Dickson

0028–0836/79/0277—0422$01.00

West Germany boosts research by one fifth

Germany is playing for a big lead in innovation, writes **Robert Walgate** from Bonn

ALMOST without debate, the *Bundestag* last month increased the budget of the West German Ministry for Research and Technology (BMFT) by 19.8% to DM5.5 billion. The emphasis is on stimulating innovation, not basic science. For example, the Deutsche Forschungsgemeinschaft (DFG), responsible for basic research in the universities, received only a 4% increase. But the ministry's programme for stimulating innovation jumped 129%.

There were also big increases in many of the directed programmes of the BMFT, particularly in electronics (32%), transport (58%), and energy, which rises 21% from DM1,720 million in 1978 to DM2,080 million in 1979. The non-nuclear research programme is growing particularly fast. It will reach DM533 million in 1979, 52% above its cost in 1978.

The increase in the transport budget represents confidence in the Erlangen Research Centre in Bavaria, where the government has already invested DM250 million in the Siemens, AEG, and Brown Boveri 450 km/h hovertrain. The investment in electronics will stimulate research in microprocessor technology.

The BMFT was not the only ministry to increase its research budget: the Ministry for Industry also announced a DM300 million boost for research in small and medium sized firms, to cover up to 30% of the cost of employing new research staff. It is hoped that this will not only correct the imbalance whereby some 90% of government-supported industrial research has been allocated to only 40 firms, but might also provide employment for the 50,000 or so un- or underemployed scientists in Germany.

Germany may be in a position of great economic strength at present, but it is certainly taking very seriously the challenges provided by its lack of natural resources, and the increasing industrial competition from Third World countries with their low labour costs. Germany seems determined to invest effectively in its brains. However there may be a more immediate reason for the success of the BMFT in this budget. The previous minister, Hans Matthoefer, is now Finance Minister in overall control of the budget, so Volker Hauff, the 38-year-old economist who now runs BMFT, has had a sympathetic ear.

However, the *Bundestag* itself showed little interest in the developments. Despite a two-day debate (23–24 January) on the whole German Budget, only the last 90 minutes (from 10.30 pm to midnight) were devoted to the affairs of the BMFT. A leading commentator on science politics in West Germany, Hartmut Altenmueller, wrote: "The interest that politicians and the public take in science was shown by the debate in the *Bundestag* last week. Zero." Only one parliamentarian, says Altenmueller, criticised the situation. This was Herr Laermann of the Centre Party, FDP (which has been taking an interest in ecology and the Kalkar fast breeder reactor lately). Laermann argued that it was time the *Bundestag* took an interest in the politics of science and technology before the government had formed its policy, made its mistakes, and presented the *Bundestag* with a *fait accompli*. But little attention was paid to his speech.

Technology minister Volker Hauff

On a longer perspective, however, German science politics is becoming increasingly interesting, and it would seem impossible that the *Bundestag* could ignore the issues. For example, Professor Helmar Krupp, Director of the Institut für Systemtechnik und Innovationsforschung (ISI), believes that there is a growing awareness in Germany that technology must be looked at through the eyes of labour: what is it like to work with technology, what influence does it have on life? Moreover, this concern, he feels, is not the exclusive property of the ruling SPD coalition. "The awareness is greater here in Germany that we must rely on high technology, that intensive work with raw materials will go out".

The government is increasingly turning to institutes like his own (it is similar to the Science Policy Research Unit at the University of Sussex in the UK) to ask for expert advice in matters of science and technology policy. For example, the DM300 million programme for stimulation of research in small and medium sized industry was designed within ISI in close cooperation with the ministry.

The energy issues, says Krupp, are much the same in Germany as elsewhere. But in basic science, Germany is facing some interesting questions. The Max Planck Institute for Plasma Physics in Garching, near Munich, is recovering rapidly from the blow of losing the joint European Tokamak (JET) project to Culham in the UK. It is now forming a proposal for a device not dissimilar in price to JET itself.

JET was delayed for some years by Brussels politics, and in some respects its design is dated. The Garching project, costing DM280 million, would be designed specifically to achieve nuclear ignition in the plasma, whereas JET is primarily a device to test scale-up (though it may also achieve ignition). The Garching device would be designed to take account of the radiation hazards.

The proposal is not yet finalised even within Garching, because one of the divisions (high beta plasmas) would have to close. But it is the main contender for Garching's main programme after ASDEX (a device under construction to test the concept of a 'divertor' to clean impurities from the edge of the plasma column). The ignition device would probably be a collaboration between Garching and MIT in the US. The view of the BMFT is not known, but, says a physicist at Garching, "at least they haven't stopped the study".

Another issue which seems sure to come to a head soon lies in a conflict between the basic research funding body DFG and the ministries concerned with a government special programme on 'health research'. Officials at the DFG are worried that criteria used for selecting research for funding under the independent, DM450 million five-year health research programme are too lax. "There is a problem of inflation of poor projects", said one DFG official. "Projects are defined loosely, and so are the assessments". Furthermore the ministries reserve the right to ignore the advice of the scientists they appoint to do the assessments, so that poor research can be funded purely on political grounds.

More recently the DFG has been asked to set up a committee to advise the government on cancer research. It may well be in that committee that the issue will be raised. ☐

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US at sixes and sevens over Soviet exchanges

LAST week, Dr Frank Press, the US presidential adviser on science, left for Moscow to discuss the progress of US-Soviet scientific cooperation.

The visit, as part of the regular programme of scientific cooperation inaugurated by the Nixon-Brezhnev agreement of 1974, was originally scheduled for last summer. It was cancelled at the last minute by President Carter in protest at the trial and conviction of Dr Yurii Orlov, the physicist, for human rights activities.

The Soviet science planners clearly welcome the restoration of full scientific relations with the US. Writing in *Izvestiya,* Dr Gvishiani, Deputy Chairman of the State Committee for Science and Technology stressed the "mutual benefit" of such exchanges and answered Western attempts to impede them. He maintained that technology transfer was not as is frequently alleged, a "one way flow"; several US firms, he said, were benefiting from Soviet technology.

At present, except for a few hardliners, there is little support among Western scientists for out-and-out boycott of the Soviet Union. Most would prefer to avail themselves of the opportunities provided by conferences in the Soviet Union to visit those of their colleagues who are dissidents and refusniks.

During 1978, however, some US academics claimed that the State Department and other relevant departments and agencies of the US government appeared to be implementing a policy of discouraging US scientists, who were visiting the Soviet Union as part of the official exchange programme, from contacting dissidents and refusniks.

It appears that pressure ranged from "friendly warnings" to the dismissal of certain known friends of the Soviet "opposition" from honorary but prestigious US government consultancies.

At the end of last year, the Committee for Concerned Scientists Inc., one of the most active human rights pressure groups, made representations to the State Department that this practice should cease. The committee was assured that no such policy in fact existed; if any such actions had been taken, it was on the mistaken initiative of individuals.

One result of the confusion of official US policy on the matter is that, on occasion, the Soviet hosts have been able to exert pressure on visiting scientists to stay away from opposition circles. In one case, a party of scientists was informed by the head of the Soviet welcoming committee that he had an "agreement" with the head of the US exchange programme in physics that scientists on official exchanges should not visit the Sunday seminar for refusniks. Challenged on the matter, the US official in question denied the existence of any such "agreement", as did also the National Academy of Sciences and the various government agencies in charge of exchanges.

If the idea of general boycott is abandoned, scientists concerned about human rights issues could take action by "working to rule" on the vexed question of substitutes. For many years Soviet delegates invited to attend conferences outside the USSR have been replaced by scientific nonentities. Originally, this resulted in free periods in the time-table, but gradually over the years it has become increasingly common for the substitutes to be accommodated in the programme.

Last summer, at the Paris conference on luminescence, a special "Round Table" on the Orlov case was held, and the possibility of a campaign against substitutes discussed. The consensus of opinion, however, was that it would cause too many practical difficulties, and possibly lead to Soviet withdrawals.

So far, only a few hard-liners, such as Nobel Laureate Paul Flery and his four co-protesters, who refused to attend the IFPAC seminar on Macromolecular chemistry in Tashkent, are prepared to make a stand on the "substitutes" issue.

However, a disturbing document issued recently by the Committee of Concerned Scientists may well make it necessary to reappraise the whole problem of substitutes. The report, alleging wide-spread anti-Semitism in Soviet mathematics, stresses that it is virtually impossible for Soviet Jewish mathematicians to travel abroad to conferences. This, it must be stressed, is not a matter of Jews who wish to emigrate to Israel, but simply of Soviet citizens with no "Zionist" inclinations who have a Jewish name and the entry "Jew" on their internal passport.

The Committee for Concerned Scientists strongly urge a "no-substitutes" policy as the best way of helping in such cases. Other countries of the Comecon bloc are, in the main, somewhat more lenient about foreign travel —with the notable exception of conferences held in Israel, which, since 1967, has had no diplomatic relations with any Comecon country except Romania. Poland is perhaps the most lenient of the Comecon countries. A handful of Polish ecologists managed to attend the Intecol-2 conference in Jerusalem last September. However, members of the Polish Biochemical Society are not prepared to rely on this precedent. Already they are making known abroad their wish to attend the meeting of the Federation of European Biochemical Societies in Jerusalem in August, 1980.

It is not sufficient, they stress, that one or two leading Academicians should attend as official delegates— what they are seeking is that any scientist from the Comecon bloc, willing to travel at his own expense, should be permitted to do so.

Vera Rich

Moscow's growing pollution problem

INDUSTRIAL pollution in the Moscow region will increase by 150–200% by the end of the century, according to Dr Aleksandr M. Ryabchikov of Moscow State University. Already the city has spread across the ring road originally intended to confine it, and new developments are penetrating into the 'green belt' economy—dairy farming, market gardening, leisure zones and reservoirs, of what is described as the 'second peripheral belt'.

Purification installations are compulsory for all new industrial enterprises, but, according to Dr Ryabchikov, are "insufficiently advanced" and are very expensive, costing up to 40% of the capital cost of the plants. Only the use of abundant non-polluting energy— thermonuclear fusion and solar energy based on photochemistry, he said, can provide a lasting solution to the problem.

Dr Ryabchikov's suggestions follow a broad-based research programme on environmental measures recently been carried out in the geographical faculty at Moscow University.

Moscow: Time for a clean-up

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Sigvard Eklund: the uses and abuses of nuclear energy

• The director-general of the IAEA (above) put the pro-nuclear case in London last week. Judy Redfearn reports

THE increased use of nuclear power has "not contributed to horizontal proliferation and is unlikely to", according to Dr Sigvard Eklund, director-general of the International Atomic Energy Agency (IAEA). At the presidential lecture of the British Institution of Nuclear Engineers in London last week, he supported this claim by saying that only six states have exploded nuclear weapons although many more have the technical know-how to do so.

The political will of states is the key to controlling proliferation, according to Eklund. Without it, international safeguards cannot be effective and "technical examinations are valueless".

Restricting the supply of enriched uranium and nuclear technology to developing countries, as advocated by the United States, is only likely to "encourage nuclear self-sufficiency and therefore increase proliferation", Eklund claims. He suggested the IAEA could work out a scheme to assure countries of essential nuclear supplies. This would have the strength of current non-proliferation arrangements and would assure supplies and safeguards. A disruption in supplies would be a breach of the safeguards. There was a need for urgent action, he said. "Nations would gain more in partnership than in imposing their thoughts on others."

Predictably Eklund is a staunch nuclear supporter. The nostalgia he feels for the time when nuclear engineers were left alone to solve difficult problems "quickly" and impossible ones "with time" illustrates his attitude towards the arguments of the anti-nuclear movement. "Rational arguments", he says, "no longer carry any weight."

Eklund believes that nuclear power will be needed to meet increasing world energy demand. He is sceptical of energy forecasting however: the great variety of estimates of future energy demand are not surprising, he says, considering the many different assumptions energy forecasters have made.

Nevertheless, he believes that the growth in demand will continue over the next decade (though the rate of growth will fall). Even if the developed countries use conservation measures, he says the world demand will continue because of increasing population, economic development, the development of sparsely populated areas (such as Siberia) and the energy needed to recycle increasingly scarce natural resources. "Even if the developed countries have zero energy growth, demand from developing countries will lead to a substantial growth in world energy needs."

Moreover, he says, conservation takes too long to have an effect to be a major factor in reducing energy demand before the end of the century. "Lifestyles and social structures don't change overnight, at least in democratic systems". Neither does he foresee any major role for alternative sources of energy. Those with the greatest potential such as hydroelectric power, he says, are already being exploited and others probably won't have much to offer.

So, in the expectation of increasing demand, Eklund sees nuclear power as ripe for a substantial contribution—at the right stage of development and economically competitive with other energy sources. "I don't believe uranium prices will rise faster than oil prices", he says, and substantial new discoveries of fossil fuels could only postpone the need for nuclear by a few decades. Oil is likely to become increasingly scarce and costly and a rapid expansion in coal production would lead to "serious environmental problems."

Eklund puts a lot of emphasis on fast breeder reactors which, he says, could double the energy potential of uranium and provide the world with abundant energy. Since 1973, however, there has been a drop in the number of FBR projects. "This constituttes a paradox", says Eklund. "Nuclear power has become the victim of the pioneering work on the environment done by its founders." The anti-nuclear movement has become allied to political groups which search for a better 'quality of life'—a term never defined, according to Eklund—rather than a higher 'standard of living'. These groups are not found in totalitarian states, he says: their greatest influence has been in countries with low majority governments such as Sweden and Austria. ☐

Can multinationals aid development?

A RAPID technological transformation, similar to the industrial expansion of Japan in post-war decades, is now facing several developing countries scattered round the globe, according to Dr Jorge Katz, director of the Economic Commission for Latin America's research project on technology and development. Indeed, these nations—which include Brazil, Argentina, Mexico, South Korea, Singapore, and perhaps even India—may soon be in a position to export advanced technical programmes to other countries—although their spread will be on more limited industrial fronts than Japan's.

The slower-developing nations of Central and South America, and also Africa, then become the main targets for industrial and technological exports from their more advanced neighbours. The question then is what these less sophisticated nations will gain in the transaction.

It is generally assumed that developing countries which are used as the sites for the advanced industrial complexes of multinational corporations gain little in the way of technological knowledge of expertise. But Dr Katz challenged this view at a recent symposium at Sussex University, organised by the Science Policy Research Unit there. He maintained that developing nations were able to assimilate information from sophisticated manufacturing plants in their midst.

"You cannot take a piece of technology from a developed country, put it in a developing country and expect it to work in exactly the same way", he said. Altered circumstances often led to changes in plant design and in this way, local engineers and technologists became involved in work that increased their expertise.

However, a variety of economic factors affected the nature of technological transfer gained at plant level. One important determinant was the amount of freedom given by multinational companies to their foreign subsidiaries. For instance, many firms in the United States allowed their subsidiaries to vary original designs, while European, especially Swiss and German, companies tended to forbid this.

Other determinants included the rate of capital subsidy; the rate of exchange; and the cost and variability of local skilled labour. In some parts of Africa, there was practically no skilled labour available. This handicapped the setting up of new factories and in turn prevented new expertise being generated. **Robin McKie**

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news in brief

WHO endorses word wide barefoot doctor campaign: The Executive Board of the World Health Organisation has approved a plan for radically improving health care throughout the world by the year 2000. Described as "a landmark in public health history" the plan addresses the failures of present medical establishments to provide satisfactory health care for the great majority of people and proposes the use of appropriate technology, improved nutrition, increased attention to water supplies and the greater use of health auxiliaries, the barefoot doctors pioneered in China.

The WHO proposals stress self reliance and technical cooperation between third world countries. According to a WHO spokesperson the plan calls for a shift away from dependence on transnational drug companies—at present countries spend up to one half of their health budgets on "useless" drugs—and away from reliance on "the great white experts" from Europe and the United States. The board also stressed that good health cannot be separated from economic and social development.

Joint UK-US reactor safety research planned: A joint testing programme on the safety of plutonium fuel will be signed shortly by the UK and the US. The programme will make use of the UK's 250 MW prototype fast reactor at Dounreay and the United States transient reactor test facility (TREAT) located at Idaho Falls, Idaho. Plutonium fuel pins will be burned at Dounreay for varying periods up to their lifetime of two years. After a cooling off period, the pins will be transferred to the TREAT facility where, singly and in bundles, they will be subjected to rapid excess reactivity. This procedure generates rapid temperature changes in the pins and is a way to simulate loss of coolant or control rod malfunction accidents. The pins can then be analysed for structural soundness against this type of accident.

The magazine, *Undercurrents* recently reported that water was found coming out of a 4 inch crack in a primary cooling pipe at the Duane Arnold nuclear reactor in Palo, Iowa. Upon inspection all seven of the cooling pipes were found to be cracked. Two plants of similar design in North Carolina have been shut down for inspection and at least nine others are at risk. A complete break in one of these pipes would cause a major loss of coolant accident of the type that the TREAT facility means to test.

US Congress elects liberals to key science policy committee posts: In a major upset to the traditional seniority system, the US democratic members of Congress have elected liberal candidates to two key subcommittee posts on scientific and environmental issues over the heads of more senior contenders.

Representative Henry Waxman of California, who was only elected to Congress in 1974, has been chosen to succeed Representative Paul Rogers as chairman of the Health and Environment Subcommitee of the House Interstate and Foreign Commerce Committee.

This committee has been the source of much recent health research legislation, as well as of concerted attempts to produce legislation covering recombinant DNA research. Mr Waxman defeated the more senior representative Richardson Preyer, who had aroused certain suspicion through holding financial interests in various pharmaceutical companies, an area of the subcommittee's responsibility.

A further surprise was caused by representative Toby Moffett, another post-Watergate congressman, who was elected to chair the Subcommittee on Environment, Energy and Natural Resources of the House Government Operations Committee.

The two appointments represent victories for labour, consumer and environmentalist interests. However, with a new generation of congressmen arriving in Washington bearing a considerably less liberal outlook than some of their immediate predecessors, few are expecting any radical shift in the activties of etiher subcommittee.

Short term contract researchers meet in Manchester: Research workers from the University of Salford, Manchester University and the University of Manchester Institute of Science and Technology met on 31 January to discuss problems of short term contract workers. Participants discussed individual cases, including that of David Pillinger (25 January, page 255) a tenured cancer researcher made redundant at the Christie Hospital: present deficiencies in the conditions of service of short term contract workers; and long term prospects including a proper career structure and strategies for long term funding including the possibility of prying money out of the University Grants Committee to fund research workers instead of just equipment and buildings as is presently the case. According to convenors Jean Shaoul and Alastair Risk of the University of Salford the questions raised are "fairly standard union matters" but the easy money of the 1960s has spoiled researchers with the result that "present organisation is well behind the rest of the working population." The next meeting is scheduled for 7 March at Manchester University.

Refusnik resists move into secret work: Since he applied to emigrate to Israel in 1974, the position of Professor Solomon Alber, a topologist working at the Institute of Chemical Physics of the Soviet Academy of Sciences has been anomalous. Normally, any such applicant is immediately dismissed from his job; Alber, however, was simply demoted from head of the mathematics department of the institute to a minor academic post. Recently, attempts have been made to shift him to the branch of the institute doing secret work—a ploy which would effectively obviate his chance of ever receiving a visa. Alber is resisting the move. If, however, he fails to comply, the institute will have some basis for his dismissal other than that of his wish to emigrate.

GE researchers develop plan for chemical heat pipe: A research team at General Electric's Research and Development Centre in Schenectady, New York has produced a feasibility study for using industrial waste heat. The study is based on an original German proposal in which heat is used to drive the catalytic conversion (at 1900 °F) of methane to carbon monoxide and hydrogen, $CH_4+H_2O \rightarrow CO+3H_2$. The reaction products are then cooled (the heat being used to warm incoming methane) and are piped over distances up to 300 miles where they are re-combined catalytically in the exothermic reverse reaction, the heat being used locally.

GE scientists sought to find a reaction that would go at the lower temperature of 1000°–1200 °F generated in many types of power station. They found a candidate in the catalytic conversion of cyclohexane to benzene and hydrogen, $C_6H_{12} \rightarrow C_6H_6+3H_2$. The efficiency of the heat conversion and extraction is estimated to be 80% but there are undetermined particle losses in the catalytic reaction. A loss of 1% per cycle would make the proposal unworkable. Research is proceeding to determine this factor.

0028–0836/79/0277—0426$01.00

A modest contribution to security and cooperation

An international science forum is to be held in Hamburg a year from now, in the wake of the signing of the Helsinki Agreement (right) John Ziman, professor of physics, Bristol University, discusses his hopes for the meeting.

TUCKED away among the fifty pages of the Helsinki Agreement is a short paragraph envisaging a 'scientific forum'—"a meeting of leading personalities in science from the participating states". In the two years after Helsinki, this item seems to have been given little attention. But amidst the wrangling at Belgrade last year, it was a relief to agree to set up an innocuous meeting of experts to plan the forum.

This meeting took place in Bonn, during the summer. For six whole weeks, diplomatic and scientific representatives of the 36 signatories of the Final Act of the Conference on Security and Cooperation in Europe (CSCE—alias the Helsinki 'Agreement')—circled warily and delicately around this modest-seeming proposal. Their eventual report was unanimous: the scientific forum will be held in Hamburg, in February 1980.

From the diffuse agenda proposed in this report it is difficult to estimate the significance of the forum. Two weeks of discussions of "interrelated problems of common interest concerning current and future developments in science" are not likely to throw much new light on the human condition, especially when dispersed over such a diversity of topics as "alternative energy sources", "food production", "trends in medical research, in particular in basic research and primarily on cardiovascular, tumour and virus diseases, taking into consideration the influence of the changing environment on human health", and "comparative studies on social, socio-economic and cultural phenomena, especially the problems of human environment and urban development".

In the diplomatic bargaining that produced this list, it was obviously easier to achieve a consensus by including the favourite topic of every delegation than to spend another six weeks trimming it down to size. Thousands of man-weeks of serious discussion of all these topics is already taking place each year, uninhibitedly across all the frontiers of the world; nobody is under any illusions that the forum can do much more scientifically than skim lightly over very familiar ground.

The original text of the CSCE Final Act intends that the forum should "promote the expansion of contacts, communications and the exchange of information, between scientific institutions and among scientists". But here again, a meeting of this kind can do little to supplement the existing network of bilateral and multilateral agreements, involving national scientific organisations, transnational scientific unions, and international agencies, under which such activities already take place on a very large scale. In this respect, the further implementation of the Helsinki Agreement to improve cultural and scientific contacts depends essentially on the amelioration of the general political climate and on detailed attention to a multitude of small obstacles to free traffic in ideas and people.

Nevertheless, those of us who took part in the Bonn meeting came to appreciate the value of the proposed forum as a symbol of the Helsinki spirit, with a modest potentiality for good, both for the progress of science and for the welfare of the people of Europe. In the acrimonious aftermath of Belgrade, it was natural that the proceedings at Bonn were ponderous, and the agreed agenda turned out to be rather trite. This was a diplomatic meeting, with all the rigmaroles of achieving complete agreement amongst the representatives of sovereign states.

If the scientists had been left alone to plan the forum, they might not have been quite so sensitive to the protocols of arriving at consensual proposals within the interlacing alliances of the 'nine' (the EEC) and the 'fifteen' (NATO). Even where there is sharp conflict of interest, as between the countries of the Soviet bloc and the Western democracies, scientific leaders can usually rely upon the shared experiences and common goals of research, so that negotiations of this kind are seldom conducted at arm's length.

But behind the courteously meandering debates and apparently trivial verbal amendments of text lay real issues, which might well have been compromised in a less self-conscious bargaining process. What is science? Is it the technological arm of state power, or does it transcend national frontiers? Should those 'leading scientific experts' who are to participate so gravely in the forum be cast as official representatives of their respective governments, or should they appear as individuals supported by the authority of their scholarly achievements? Are the topics to be treated largely from the aspect of practical application—hence within all the constraints of economic, political and military competition—or in those fundamental aspects that are universal and free for all mankind? These questions were a constant preoccupation of the diplomatic and scientific representatives at Bonn, on both sides of the Great Divide.

On the whole, the agenda of the forum leaves the door as open for truth as for the manifestations of power. This is something of an achievement. From the beginning, the Soviet government and its closest allies were fearful that the West might be tempted to use the forum as an instrument of protest against the oppression of dissidents. Their desire to impose official representative status on the speakers at the forum was as much an attempt to muzzle such 'undiplomatic' mouthpieces as a reflection of their own heavily bureaucratised and subservient scientific systems. The Bonn report does not impose such a gag, thus effectively recognising the universality of the voice of science.

In my view, it would be a mistake for Western scientists to press the human rights issue at the forum. This I say reluctantly, for this issue is profound within modern science. But what is offered at Hamburg, in little more than a year's time, is the opportunity to celebrate, under official governmental auspices, as publicly and as explicitly as could be, the genuinely transnational character of the scientific enterprise. It will be an occasion to demonstrate the universality of empirical fact and rational argument, the communal spirit that pervades research work in all fields, the fraternity and cosmopolitanism of the republic of learning, and the humane benefits that accrue from a body of reliable scientific knowledge. These are the norms to which we refer, these are the ideology of science itself—the ideology to which the dissidents themselves appeal in their opposition to tyranny.

The Helsinki Agreement was about cooperation and détente. The true meaning of these unhappily tattered concepts in the scientific and cultural domain must not be forgotten. The value of the scientific forum is unlikely to be found in deeper understanding of technical scientific issues, nor in another confrontation on socio-political fundamentals, but perhaps in a modest affirmation that truth itself is one of the powers to be reckoned with in the real world. ☐

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correspondence

Causes of cancer

Sir,—In a recent book review I stated that "many, and perhaps most, cancers are caused by certain sexual habits, smoking habits, and gross aspects of diet rather than contaminants" (27 July, page 404). My statement runs counter to the general belief that we are currently suffering an epidemic of cancer due to a multitude of carcinogenic pollutants, and I have been asked, both courteously in private correspondence and sarcastically by Ralph Chernoff (7 September, page 8) to substantiate it. Chiefly, those who are surprised by this statement should read the relevant reviews of cancer epidemiology (*Nature* **265**, 589; 1978) or of cancer trends, but some relevant points can be summarised briefly.

The major age-specific trends over the past decades are not in general upwards, with the notable exception of those cancers associated with cigarette smoking. The American Cancer Society (*A Journal for Clinicians*, **28**, (1), 20–21; 1978) have illustrated this for America, and Doll (*Proc. Roy. Soc.*, in press) has shown it in more detail for Britain, avoiding the older age groups where the progressive elimination of mis-diagnosis produces artefactual trends. Any balanced perspective on cancer must begin with the role of smoking, not with any other aspect of the modern world. (The arguments recently advanced by the office of OSHA in the US that 20–40% of all cancer will soon be caused by industrial exposure are methodologically unsound, for the particular risks which they discuss are very considerably exaggerated.)

Smoking, particularly of cigarettes, is the chief cause of lung cancer in Britain, and smoking is strongly associated with oral and with oesophageal cancer and also, albeit less strongly, with bladder and with pancreatic cancer. Adding up appropriate proportions of the numbers who die of these cancers, smoking is responsible for about 30% of all British cancer deaths, most of this 30% being accounted for by the solidly established causal relationship betwen smoking and lung cancer.

Sexual habits are the chief known determinant of the risk of cancer of the uterine cervix, a disease which is very much commoner in prostitutes than in nuns and which is commoner among women who have had many sexual partners or who began intercourse at an early age. This disease, however, accounts for only 2% of all British cancer deaths, and although we have accounted for "many" British cancer deaths by smoking, we have not yet accounted for "perhaps most" deaths, and cannot do so unless we can describe likely causes for stomach, breast or colorectal cancer.

Stomach cancer used to be very common in the United States, but over the past forty years the mortality from it has decreased tenfold, so that it is now quite rare. The causes for this are quite obscure, especially since treatment has not materially improved, but since downward trends in stomach cancer are at last also developing in Britain one can hope that whatever (dietary?) aspects of the twentieth, as opposed to the nineteenth, century environment has protected the Americans will also eventually protect us.

In Britain, apart from lung (27% of cancer deaths) and stomach (9%), the two commonest sites are breast (9%) and large intestine (13%), and preventable causes for these definitely exist although they cannot as yet be identified with certainty. Early pregnancy seems to protect against breast cancer, but no practicable other way of producing these protective effects is yet known, so let us turn to diet instead. Among rats that have been pre-treated with certain carcinogens, a moderate dietary increase or decrease of certain components of "fat" will greatly increase or decrease their chances of developing breast or colon cancer. In humans, graphs of the per caput fat consumption in various countries against age-standardised cancer rates have been found to yield very striking positive correlations with fat for female colon cancer (81% correlation), for male colon cancer (85%) and for female breast cancer (89%). These correlations, taken together with the evidence in laboratory animals, make it plausible that some gross aspect of diet is a preventable (since people already differ with respect to it) cause of these cancers.

Of course, one particular person's fatal cancer may have had more than one preventable cause, especially if multistage models for cancer induction are approximately correct (Peto, 1977, *Origins of Human Cancer*, Cold Spring Harbor publications). However, this makes it more, rather than less, plausible that among "sexual habits, smoking habits and gross aspects of diet" (such as the consumption of certain fats or fibres) we shall find at least one preventable cause for many, and perhaps most, of today's fatal cancers.

Yours faithfully,
Richard Peto

Radcliffe Infirmary
Oxford, UK

World futures

Sir,—I would like to point out some curious omissions in Lord Ashby's review of Freeman's and Jahoda's "World Futures" (9 November, page 144).

Ashby cites with approval the choice of four growth options; he forgets that there are also four decay options, which should not be left out *a priori*, merely on the ground that they are less cheerful than the former.

When Ashby agrees with the "outstanding practical conclusion" from the analysis, namely that the absolutely top priority is to narrow the gap between rich and poor, both within and between nations, and that this can only be done by devoting massive resources to certain problems, he fails to mention two important facts. First, that this narrowing of the gap between rich and poor is exactly what has been happening in Western industrialised countries for the last hundred years; second that the same thing is happening now between nations, without any wilful action at all. Although most poor nations have not become richer, western European countries have become immensely poorer, through the operation of political, economic and social forces, such as displacement of industry, loss of control over resources and the corresponding inflation. If this trend continues, these nations will be fully-fledged members of the Third World within a very short time, and this would of course resolve the problem of their devoting massive resources to aid other countries.

Yours faithfully,
S. V. Vaeck

Ministère des Affaires Economique,
Brussels, Belgium.

Energy forecasts

Sir,—Your correspondent reports Amory Lovins commenting favourably on the predictive power of his tabulation of US Energy Demand Forecasts, (18 January, page 163). My analysis supports the opposite view.

Neglecting Steinhart's forecast for 2050 as outside the boundary, and taking instead the lower CONAES 2010 figure as "beyond the pale" for '78, you will discover that the range between maximum and minimum for each year of forecasts is nearly constant at 60 q. Thus as the year of forecast approaches the target year 2000, so the percentage error on the forecasts increases! Is this knowing more and more of less and less?

Yours faithfully,
J. T. D. Mitchell

Abingdon, Oxfordshire, UK.

ARMS cannot negotiate

Sir,—We were gratified to read Mr Hounsell's letter (18 January, page 171) and his expression of support for ARMS albeit with reservations. Let us allay his fears on one or two points. First, the prime objective of ARMS is to seek to develop and establish career structures for full-time research scientists in biomedical sciences, and who could be better motivated than the scientists themselves to do this. Second, we recognise that we have neither the experience nor the rights to 'negotiate' with employers (in the trade union sense) but we can at least enter into discussions, identify problems and make recommendations to both employers and the unions who aim to represent us. If researchers become diverted from working within a trade union as a result of this, then it can only mean that the unions themselves are failing to fulfil the requirements of these workers.

Yours faithfully,
Arleen Unger

ARMS, Clinical Science Labs.,
Guy's Hospital, London, SE1.

news and views

Synthetic genes for human insulin

from Michael J. Gait

THE synthesis of human insulin by bacterial cells fermented on production scale, a dream of molecular biologists for several years, is rapidly approaching reality with the recent announcement of the expression of synthetic human insulin genes in *E. coli* (Crea *et al. Proc. natn. Acad. Sci. U.S.A.* **75,** 5765; 1978; Goedell *et al. Proc. natn. Acad. Sci. U.S.A.*, in the press).

The dream began early this decade with the discovery that pieces of DNA of different origin could be inserted into a bacterial plasmid or virus (*in vitro* recombination). The chimaeric DNA could be used, for example, to transform *E. coli* and clones containing the desired insert selected. It was not difficult to imagine that one day DNA coding for insulin could be inserted into a bacterium and expressed as mature insulin. The idea was also attractive to pharmaceutical industry, conventional methods of insulin production being hopelessly inadequate for projected future needs.

Recently several giant strides have been taken towards insulin expression in bacteria. First, rat pre-proinsulin mRNA has been reverse transcribed into cDNA and inserted into the plasmid pMB9, which was used to transform *E. coli* cells. In one clone the whole proinsulin coding sequence and part of the N-terminal pre-peptide was present (Ullrich *et al. Science,* **196,** 1313; 1977; see also *News and Views* **268,** 198; 1977). More recently Villa-Komaroff *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75,** 3727; 1978) inserted cDNA, again made from rat pre-proinsulin mRNA, into the coding sequence for the secreted protein penicillinase located on the plasmid pBR322. After transfection and cloning a fused penicillinase–proinsulin translation product was detected to be secreted from the bacterial cell. Isolation of human pre-proinsulin mRNA is technically difficult and has not yet been accomplished. Fortunately an alternative and potentially much more far-reaching approach is now possible based on the use of chemically synthesised DNA.

As a curtain-raiser K. Itakura *et al.* (*Science* **198,** 1056; 1977) showed that synthetic DNA corresponding to the sequence of the peptide hormone somatostatin could be inserted into the plasmid pBR322, this time into the gene for the secreted protein, β-galactosidase. An extra methionine codon was incorporated at the N-terminus of the somatostatin gene sequence, so that after expression in *E. coli* the secreted, fused, β-galactosidase–somatostatin product could be cleaved at the methionine residue by treatment with cyanogen bromide, liberating somatostatin (which contains no methionine).

The same research group has now described the chemical synthesis of two pieces of DNA corresponding to A and B chains of human insulin (Crea *et al. op. cit.*). Each DNA duplex was separately inserted into the plasmid pBR322 in the same way as for the somatostatin gene. After transformation of *E. coli,* fused β-galactosidase-insulin A in one case and β-galactosidase-insulin B in the other were found to be secreted (Goedell *et al. op. cit.*). Free A and B chains are generated by cyanogen bromide treatment, but so far only A chain has been isolated in reasonably pure form. Nevertheless, yields of insulin chains are about 10 mg per 24 g wet cells, about 10 times higher than for somatostatin.

The aim is to reconstitute mature insulin by association *in vitro* of disulphide bonds between A and B chains (proinsulin *in vivo,* consisting of B chain, a connecting peptide and A chain, folds and forms two disulphide bonds between chains A and B. The connecting peptide is then excised to generate mature insulin). One drawback to synthesis of separate insulin chains is that the achievement of high efficiency of disulphide bond association in the correct orientation is currently disputed amongst specialists. Goedell *et al.* (*op. cit.*) indeed report low yields when impure A and B chains are used and so far have only detected mature insulin by radioimmunoassay. A better approach might be to synthesise the DNA sequence corresponding to proinsulin or an analogue with an easily excisable connecting peptide. Here disulphide bond formation *in vitro* is likely to be much more efficient because A and B chains are already in close proximity. However, chains of a possibly active human gene in *E. coli* would require much more stringent containment under the present guidelines than the P2-EK2 used to clone the chains separately. The safety of this procedure would need to be established before large scale cloning could be considered.

Despite the uncertainty of insulin reconstitution the methodology as it stands must be recognised as a remarkable contribution to molecular biology, at least as important as the pioneering synthesis of RNA genes by H. G. Khorana's group (see Khorana *et al. J. biol. Chem.* **250,** 565; 1976; the synthesis of the tyrosine suppressor tRNA gene has finally been completed (T. Sekiya *et al. J. biol. Chem.*, in the press)). Khorana introduced the technology for joining chemically synthesised oligodeoxyribonucleotides 10–15 units long with the enzyme T4 DNA ligase. Using the specific base-pairing mechanism two such chains could be joined in the presence of a third 'splint' chain. A duplex is formed by simultaneous joining of several chains aligned in this way. Crea *et al.* (*op. cit.*) have used the same enzymatic technology, but the chemical synthesis of the 29 oligonucleotides needed to form the two duplexes has taken only a fraction of the time (in man-years) compared with that required for the tyrosine tRNA gene. This is the result of the use of the developing 'phosphotriester' synthetic technology (compared with Khorana's phosphodiester approach) as well as new rapid purification techniques afforded by high performance liquid chromatography.

Now that synthetic DNA can be prepared on a reasonable time-scale the

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Gravitational radiation at last?

from P. C. W. Davies

SCIENTISTS have used astronomy as a proving ground for theories of gravity ever since Newton explained the sizes and shapes of the planetary orbits. In 1915 Einstein proposed a new theory of gravity—general relativity—involving bizarre concepts of geometrically distorted space-time, and in a short while minute corrections to Newton's theory were confirmed by careful observation in the Solar System.

Until recently, no qualitatively new tests of general relativity were made. Then, with the discovery of a weird type of double star in which gravitational fields are thousands of times stronger than in the Solar System, the possibility arose of confirming subtle higher-order relativistic corrections to Newtonian gravity. Known as a binary pulsar, the new object is thought to be a pair of imploded stars in orbit around one another. One star is unidentified, but the companion seems to be a neutron star, an object with the mass of the Sun but shrunk to only a few miles in size, so that its immense gravity causes even atoms to collapse into neutrons under their own weight. Rotating many times a second, this collapsed star emits extremely regular and precise pulses of radio waves (hence the name pulsar) which can be monitored on Earth and used as a built-in clock.

Over the past 4 years astronomers have beeen able to follow the convolutions of the pulsar in its orbital dance around its invisible companion. Because of the enormous gravitational fields in the system, relativistic effects are extremely prominent. For example, a progressive twisting of the elliptical orbit caused by space-time curvature amounts to a huge 4.2 degrees per year, which should be compared with the celebrated 43 s of arc per century suffered by the planet Mercury.

On page 437 of this issue of *Nature*, three astronomers report measurements on the pulsar taken with the giant Arecibo radiotelescope in Puerto Rico. The precision of the observations and the quantity of information encoded in the radio pulses is so high that not only can the mass of the pulsar and its unseen companion be deduced, but an internal consistency check can be made on the general theory of relativity. The masses are much as expected, somewhat less than 1½ solar masses, suggesting that both components are neutron stars, but the more exciting result concerns a small, but vitally significant feature of the orbital motion. The astronomers have found that, over the months, the orbital period of the pulsar (which is less than 8 h) is slowly changing. Although the shift only amounts to about one part in 10^9 per year, it is quite distinct and has far-reaching implications.

The systematic drift is readily explained by Einstein's theory. Just as the motion of electric charges produces electromagnetic waves, such as light and radio waves, so the orbital gymnastics of the binary pulsar should produce gravitational waves. In Einstein's theory these are literally ripples of space-time itself, emanating from the central disturbance, transporting energy away from the star system into space. The energy loss is paid for by a decay of the pulsar's orbit, and it is this which the astronomers seem to have spotted. It is similar to deducing the existence of radio waves by observing the rise in one's electricity bill when the transmitter is switched on. Although indirect, the discovery is nevertheless powerful support for the theory of relativity and is bound to attract a lot of scrutiny. Other theories of gravity exist, some of which also predict gravitational radiation, but in many of these the expected effect is considerably larger than that observed. One point which will receive attention is the fact that the relativistic formula that seems to be confirmed is actually only an approximation. The theory of relativity is mathematically extremely complicated and non-linear, and there is some uncertainty over how accurate the existing approximation really is. There is clearly room for more theoretical work here.

Gravitational waves have long been sought on Earth using metal bars or large crystals as detectors. The principle of these detectors, pioneered by Joseph Weber of the University of Maryland, is to measure a faint ringing sound as the space ripples sweep by. Gravity is so weak that even the strongest bursts of gravitational radiation expected from space will deliver less energy to the bar than would a lighted match held a million miles away. Tremendous advances in technology have been needed to overcome this daunting feebleness, and many physicists, despite some early evidence of success, believe that direct detection of gravity waves here on Earth is still some years away. However, new techniques, and new types of detector such as the laser interferometer, should eventually give birth to the new science of gravitational astronomy, enabling astronomers to look at the binary pulsar and other sources of gravity waves with gravity telescopes.

The last great test of Einstein's general theory of relativity, by Sir Arthur Eddington in 1919, caused a public sensation when it was confirmed that the Sun's gravity bends starlight. The binary pulsar has been the first opportunity for the theory to be tested beyond its first-order corrections to Newtonian gravity, but physicists are treating this latest success with quiet satisfaction. Few seriously doubted the outcome. Nevertheless, it is fitting that the centenary of Einstein's birth should see the confirmation of one of the more intriguing consequences of his work.

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particular advantages of its use can be truly appreciated. For there are no tricky mRNA isolations involved, merely a knowledge of the required peptide sequence. Codon choice seems not to be a problem, as both the human insulin duplexes (incorporating codons chosen from those most frequently found in phage MS2) and the rat proinsulin cDNA have been expressed in *E. coli*. In the future other peptides and proteins may well be prepared in this way. The DNA sequence need only be synthesised once, Nature's own peptide synthesis mechanism being useable *ad infinitum* (with careful checks for the absence of spontaneous mutations). Moreover, it would be relatively simple to incorporate changes in the amino acid sequences of a protein (by altering the DNA sequence) that might in turn alter its biological activity.

Whereas synthesis of complete genes still requires a substantial investment of finance and personnel, there are several areas of molecular biology where the availability of short, sequence-specific oligodeoxyribonucleotides has made considerable impact recently. Particularly noteworthy is the use by C. A. Hutchinson *et al.* (*J. biol. Chem.* **253,** 6551; 1978) of a 13-mer

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complementary to part of the phage φX174 sequence in all but one nucleotide corresponding to the position of the *am*3 mutant. The oligonucleotide was incorporated into a complete, circular, complementary strand, when used as a primer on wild type DNA, with the deliberate G–T mismatch causing an amber mutation in the newly synthesised strand. The use of mismatched oligonucleotide primers could in principle also be used to induce transversions, insertions and deletions, and it is likely that site-specific mutagenesis by this and other techniques will be an area of great future activity.

Synthetic oligodeoxyribonucleotides have also found increasing use in the analysis of DNA structure and function. This topic has been recently reviewed by R. Wu *et al.* (*Prog. Nucleic Acids Res. molec. Biol.* **21**, 101; 1978) and includes applications to DNA sequence analysis, the study of protein–DNA interactions, and the use of oligonucleotides as probes for genes and as tools for molecular cloning of DNA. Indeed the recent dramatic increase in the demand for synthetic DNA has only been partially met by the introduction of more rapid synthetic methodologies. Until commercial enterprise can meet this phenomenal demand some sort of DNA synthesis facility is now an important asset to laboratories involved in molecular biology. ☐

Moscow revisited—laser fusion 1979

from Ian Spalding

It is eight years since the European Conference on Laser Interaction with Matter (ECLIM) was last held in Moscow. At that time only a handful of visitors from the West contributed to an otherwise well-attended conference; the practical importance for laser fusion of creating highly compressed DT targets (with densities much exceeding those normal in the solid state) was becoming increasingly stressed in the published literature, but the available laser technology was still relatively primitive. As a particular example, the energy then available from a state-of-the-art pulsed CO$_2$ laser was only 10 J, with a peak power of ~10 MW! Of comparable importance, the absorption coefficient for radiation of wavelength λ between $\frac{1}{2}$ and 10 μm as a function of incident angle, polarisation and intensity, and as a function of plasma composition and initial distribution, had not been characterised in systematic experiments—nor were the existing measurements well coordinated with available theoretical (mostly analytic) models of relevant linear and non-linear interactions. Although no fundamental break-through was reported at the twelfth conference in the series held recently*, overall progress in the field has been significant and encouraging.

Advances in laser technology over the past eight years have been particularly striking. 10 kJ, 27 TW, Nd : glass and 10 kJ, 20 TW, CO$_2$ laser systems have been successfully commissioned in the past year at the Lawrence Livermore and Los Alamos Scientific Laboratories respectively; all of the 1 μm and approximately a quarter of the 10 μm power has already been symmetrically focused onto gas-filled microballoon 'exploding pusher' targets. DT neutron yield increases with incident Nd laser power to an (unoptimised) 3×10^{10} neutrons at 27 TW; scaling at both wavelengths fits quasi-analytic models. Significant thermonuclear burn will however require the experimental achievement of an isentropic compression of the target's core, so more complex layered microballoon 'ablative burn' targets are now receiving increasing experimental attention. At incident powers $<10^{12}$ W, the KMS and KALMAR (Lebedev) Nd lasers have demonstrated compressions approaching × 35 (hydrogenic) solid-state densities, using cryogenic targets and α-emission spectroscopy KMS claim that this compression is partly adiabatic. In summary, available 'driver' powers have increased tremendously over the past eight years—for CO$_2$ lasers by a factor of 10^6; ideas advanced for larger systems (regenerative relayed amplifiers, stimulated Raman/Brillouin convertors and so on) suggest that 0.1~1 MJ drivers (predicted to be of practical interest) may well be operational within the next decade.

Developments in the understanding of laser–plasma interactions, and of the necessary diagnostic techniques, are equally impressive. Various groups have experimentally investigated the absorption of laser light at wavelengths λ of $\frac{1}{4}$, $\frac{1}{2}$, 1 and 10 μm, and at intensities I within at least part of the range $I=10^{10}-10^{16}$ W cm^{-2}; there now seems to be universal agreement that strong density-profile modifications are induced by radiation pressure effects when $(I\lambda^2)\sim10^{15}$ (W cm^{-2}) μm^{-2}, and that resonance absorption significantly increases the total absorption coefficient at the longer wavelengths. Related measurements suggest that much of the absorbed radiant energy is then converted to hot, non-thermal, electrons. Developments in two and three-dimensional soliton theory, and in analytic modelling of strong turbulence by the Russian school, are equally impressive. The main topic for controversy appears to be the computer optimisation of target designs, where the avoidance of unwanted core preheating by the 'hot' electrons and of possible deviations from the desired symmetry of implosion (due to Rayleigh-Taylor instabilities) poses significant modelling problems in the hydrodynamic codes. The US prefer 'thick' (lower aspect ratio) double-shell designs to obviate both of these problems. The increasing tendency of American and Russian theoreticians to 'cross-calibrate' predictions from their codes against published experimental work seems therefore a noteworthy and potentially fruitful development.

It is particularly interesting that Academician E. P. Velikhov took the opportunity of a round-table discussion (including proponents of electron-beam fusion and fission/fusion hybrids) to suggest that the time was ripe for a cooperative international venture in laser fusion, perhaps under the auspices of the IAEA. In making this personal suggestion, he cited the recent IAEA decision to initiate the Intor (joint-Tokamak) study as a bold and historic venture. ☐

I. J. Spalding is in the Experimental Division A, UKAEA, Culham Laboratory.

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*ECLIM XII was held in Moscow's Polytechnic Museum on 11–15 December, 1978.

Variations on a theme

from Peter Newmark

Without their analogues, synthetic or natural, insulin experimenters would soon be on the streets. Fortunately the chemist's ingenuity keeps ahead of the biologist's appetite for quantity and variety. For it is through the application of old and new analogues that those attending a recent meeting* saw the eventual solution to most of the mystery still surrounding the actions of insulin.

How exactly, for example, does insulin bind to its cell surface receptor? The combined powers of crystallography and analogue study have outlined the domain on the surface of the insulin which is implicated in binding—a domain which has never been so clear as in the computer-generated molecule presented by P. de Meyts (ICP, Brussels) and R. A. Feldman (National Institutes of Health, Bethesda). (This film was not just a

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*A European Workshop on Insulin Structure organised at the ICP, Brussels, 13–15 December, 1978 by P. De Meyts and D. Brandenburg.

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remarkable travelogue around the surface of insulin but, since its images are generated from molecular X-ray coordinates it heralds a more accurate and far easier method of 'building' models than is now possible). Although the binding domain has been identified, the specific role and importance of individual amino acids within the domain remain uncertain. Certainty may come from new analogues. Perhaps the chemists will succeed in modifying one of the implicated amino acids in a minor way but with drastic consequences for binding. Or perhaps the isolation of a natural insulin analogue from some exotic species will spare the chemist's toils.

It would also help if the receptor for insulin were available in purified form. But that, according to L. Kuehn (Dusseldorf University), will require either a method of affinity chromatography better than any now available, or an alternative, non-classical approach. Matters may be helped by the availability from the laboratory of D. Brandenburg (Deutsches Wollforschungsinstitut, Aachen) of photoaffinity insulin probes for the receptor, which are now being put to use.

Another very recent Aachen contribution is a series of covalent dimer insulins, potentially useful probes of the subunit structure of the receptor. Several varieties of covalent linkage are possible and A. Schuttler described their preparation from insulin molecules with protected end groups.

Early data from the testing of the dimers in various biological systems have contained enough oddities to arouse even the most jaded analogue addict. In the adipocyte lipogenesis assay, for example, the activity of the dimer in which the linkage is between position 1 of the B chain of one molecule and position 29 of the B chain of the other is only very modestly reduced compared with native insulin (R. Jones & P. Sonksen, St Thomas's Hospital Medical School; Schuttler). Also, and against the usual correlative trend, the activity *in vivo* on blood glucose levels of this dimer was much lower than on lipogenesis *in vitro* and, for the activity observed *in vivo*, the blood clearance rate of the analogue was surprisingly high (Jones & Sonksen).

The crystal structure of insulin is of course well established at high resolution but as T. Blundell (Birkbeck College, London) emphasised, crystalline insulin is in hexamer or dimer form which may not provide a good guide to the structure of the biologically active, soluble monomer. Early indications from a variety of sources suggest that insulin does have flexible surface groups whose structure changes considerably when insulin polymerises contacts. According to Blundell the conformational flexibility seen in the crystalline insulin hexamer is not so evident in its stable-mate, pancreatic polypeptide III, the high resolution structure of which is in the offing.

With an inevitability characteristic of any peptide hormone meeting these days, matters were not complete without the externalisation of internalisation. The most thorough contribution came from J. L. Carpentier (Ecole de Medicine, Geneva). The data he presented showed convincingly that $[^{125}\text{I}]$ insulin was internalised in lymphocytes and hepatocytes and, with time, accumulated in lysosomes and/or Golgi bodies. The 'point' of internalising insulin (and other hormones) remains enigmatic. There is no evidence for a functional role for insulin within the cell despite the somewhat surprising claim by Carpentier that the internalised hormone is largely undegraded and therefore potentially active. It must be recorded however that data from other less thorough studies was not all consistent with internalisation.

Finally J. Gliemann (Copenhagen University) showed that insulin monoiodinated at A14 (tyrosine 14 of the A chain) had twice the affinity of the A19 isomer for the receptor on adipocytes, a factor of such consequence to some experiments that they will bear repeating when the pure A14 isomer is available. Then there is a move to iodinate all analogues and to redetermine their binding parameters by direct instead of indirect means. And there are no end of interesting vertebrates whose insulin has yet to be sequenced—the latest to fall being the casiragua (R. Horuk, Birkbeck College) a rodent relative of the coypu and guinea pig. And still to be answered categorically is the question of whether invertebrates have insulin or some forerunner molecule.

Insulin experimenters, the world is your oyster. Oysters, does your world have insulin? ☐

Peter Newmark is the Biological Sciences Editor of Nature.

The world's vanishing primates

from Michael Kavanagh

THE International Primatological Society this year held its Congress in Bangalore, India and in one of the satellite symposia* attention was focused on the enormous problems facing primate conservationists in neighbouring Bangladesh. The forests that are the natural home of the country's monkeys and apes are now almost entirely restricted to three areas in the centre and south of the country and they are being destroyed at an alarming rate. None is totally protected and whereas the government estimates Bangladesh's forest area at about 8,600 square miles, this figure is reduced to less than 3,000 square miles when timber monocultures, tea estates, rubber plantations, grasslands, scrub and other man-made vegetation types are excluded. 'Common' langurs (*Presbytis entellus*) seem to be extinct and there are estimated to be only about 35,500 of the most abundant primate, the rhesus monkey (*Macaca mulatta*), left in the forests. A few more survive in the towns, but in contrast to India where wildlife is generally revered, Bangladesh is a country in which most people are ignorant about wild animals and regard them as dangerous or destructive objects to be killed or persecuted in any way possible. In describing his country's situation, Ali Reza Khan stressed habitat destruction and the lack of education about the natural heritage as the two major threats to primate conservation. Habitat destruction results from the needs of a human population that has reached an average density of 3,000 per square mile of land; and as far as education is concerned, Reza Khan reported that international help is desperately required for such basics as buying the books that Bangladesh cannot afford. He illustrated the Bengali attitude to wildlife with the story of a serow (*Capricornis sumatraensis*), a large, long-legged goat antelope, that wandered over the border from India. It was chased by a mob of thousands of people who attacked it with sticks and stones. Eventually the police intervened and took the animal into protective custody in the local jail until they could hand it over to the Forestry Department.

In describing the plight of Bangladesh's primates and in calling for a moratorium on all exports at least until the primate populations have been thoroughly surveyed (a task in which he is involved), Reza Khan reflected the major concern that dominated the congress. Although the society is an academic body, no academic issue caught the minds of large numbers of

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*Satellite Symposium on Primate Population Studies in India, Bangalore, India, 6–7 January, 1979. Part of the VIIth Congress of the International Primatological Society.

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delegates in the way that was true of conservation.

Inaugurating the congress, V. Ramalingaswami, President of the Indian National Science Academy, stated that half of the world's 180 non-human primate species were in danger of extinction and he called for a deeper understanding of primate biology in order to facilitate management for survival. He urged biomedical researchers only to use primate subjects where absolutely necessary and pointed out their common interest with conservationists in that both groups desire the preservation of primate populations.

A Conservation Round Table, which was intended to last for a morning, continued by demand of the participants throughout the afternoon with delegates offering summaries of the situation in almost every country that has indigenous primates. In summing up this session in his report to the Business Meeting of the Society, Vice-president J. S. Gartlan struck several themes. First, education about conservation appears to be a major need throughout much of the world. Where efforts have been made, the results have been encouraging. A case in point is Kenya, where the Wildlife Clubs are working successfully in schools. At a higher level, there is a need for the education of field staff (such as game rangers) in the developing countries. This must go hand in hand with the provision of incentives in terms of jobs and security.

Second, the greatest threat to natural primate populations comes from habitat destruction, with trade and hunting for any purpose being of variable seriousness in different areas. He contrasted India where wild animals are seldom molested with West Africa where hunting for protein is a major problem.

Third, the outlook in any area can change rapidly, often due to government action. For example, Guyana still contains large areas of relatively undisturbed forest in its interior, but the government has declared its intention to develop this land for human use including that of providing a site for a new national capital. Gartlan urged continual surveillance of parks and reserves to guard against encroachments or changes in their legal status.

Fourth, management of the ecosytem is essential for a conserved area, and therefore any plans to create a national park or a reserve are worthless without such management.

Gartlan's fifth theme reflected the activist mood of the delegates. He pointed out that most wild primate populations are to be found in tropical countries where much development is funded through international agencies. He suggested that modification of plans at an early stage could often be of benefit to primate conservation and that therefore the International Primatological Society should develop an

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The primates of peninsular Malaysia

from David Chivers

The evergreen rain-forest that covers nearly half the land area of Peninsular Malaysia is rich in primates and other wildlife. There are three species of lesser ape, the siamang (*Hylobates syndactylus*) and lar gibbon (*H. lar*) with the agile gibbon (*H. agilis*) in the north, two species of macaque, the long-tailed macaque (*Macaca fascicularis*) and the pig-tailed macaque (*M. nemestrina*), two species of leaf monkey, the dusky leaf monkey (*Presbytis obscura*) and the banded leaf monkey (*P. melalophos*), (with a third species, the silver leaf monkey (*P. cristata*) in the mangrove swamps of the west coast), and the prosimian slow loris (*Nycticebus coucang*).

Habitat varies with altitude; the lowland Dipterocarp rain-forest has the most varied flora and fauna, and primates abound. The gibbons and leaf monkeys extend up into hill Dipterocarp forest and sub-montane formations, but the smaller gibbons and dusky leaf monkey are more restricted to the lower forests. Pig-tailed macaques show a preference for the higher, more remote forests, while the long-tailed macaques prefer riverine forest and disturbed habitats, in which they scavenge on the by-products of human society.

Forest clearance poses the main treat to Malaysia's wildlife; timber is a valuable resource which has been over-exploited. There is increasing realisation that such benefits are short term, with possible disaster beyond. The maintenance of permanent forest reserve over about 45% of the Peninsula will, it is hoped, contain the increasing problems of flooding and soil erosion. The long-term economic benefit of sustained, light, selective logging, harvesting of other plant products, and cropping of various mammal species in such managed forests, and the benefits of protecting watersheds and organising tourism in undisturbed habitats remain to be established. This needs to be coupled with the more efficient use of agricultural areas.

Until this long-term equilibrium between man and wildlife is established there is the distressing problem of wildlife made homeless by clear-felling. Unless animals can be transferred to other forests where numbers have been reduced by hunting, they are doomed. The Wildlife Department have shown their interest in tackling this problem, and current research projects will, one hopes, provide guidance.

The Game Department in Malaysia has been reorganised into the Department of Wildlife and National Parks, with the Chief Game Warden, Encik Mohamed Khan bin Momin Khan, KMN, as its Director-General; numerous research officers have been appointed, and a generous allocation of government funds has been made for conservation in the current quinquennium under the Third Malaysia Plan. Apart from coordinating the conservation effort, with encouragement from the Malayan Nature Society, the department is also responsible to the Ministry of Science, Technology and Environment for maintaining national parks and game reserves and enforcing the rigorous laws that protect Malaysia's wildlife. Only *M. fascicularis* is exported at present, at a rate of less than 10,000 a year. More information is needed urgently to assess the effect of this on the wild population, whether the export of any other species can be justified for essential medical research and, if so, in what numbers.

It is against this background that a 3-year programme of collaborative research between Universiti Pertanian Malaysia, Universiti Kebangsaan Malaysia and the University of Cambridge was started in July 1978, assisted with funds from the US Public Health Service, the L. S. B. Leakey Foundation and The Royal Society. The main aims are to study the socio-ecology of the lesser-known species such as the slow loris and the pig-tailed macaque, and to assess the distribution and habitat preferences of each species throughout the peninsula. The intention is to determine precisely the effects of different degrees and types of habitat disturbance on primate populations. Laboratory studies on many aspects of the biology of the indigenous primates will be made using animals displaced by forest clearance. These studies should provide the information essential for their conservation in both natural and disturbed habitats.

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early warning system by monitoring international agency intentions. The possibility would then be open for members in areas to be affected to comment constructively to the benefit of conservation requirements.

Finally, he pointed out the failure of many countries to sign the Washington Convention on International Trade in Endangered Species. Austria, Italy and Belgium have not signed, and as a result Europe is a major centre for the smuggling of endangered primates. Similarly, Japan has failed to sign and, according to Gartlan, is a major market for endangered species.

Many delegates volunteered to participate in a network of primatologists who may be called on for a coordinated letter-writing campaign whenever a conservation crisis threatens. The hope is that evidence of worldwide concern will persuade national agencies to modify development plans that threaten primate populations. In my experience, conservation is always a major issue at meetings of the International Primatological Society, but never before has there been such a general sense of urgency. ☐

Compensation of Doppler-broadening using light shifts

from Peter Knight

A NOVEL technique has been developed by Cohen-Tannoudji and his colleagues at the Ecole Normale Supérieure and Collège de France to produce Doppler-free optical emission with highly anisotropic spectral properties. Normally a cell of excited atoms in random motion will produce an optical field by spontaneous emission whose spectrum is isotropic: no matter which end you look at with a spectrometer, the radiation will be seen to be broadened by Doppler effects to a width typically many times larger than the natural single-atom width. Cohen-Tannoudji has found ways to pump a cell of atoms in random motion with an intense laser field so that the atom can emit (or absorb) radiation without Doppler broadening. The radiation from such pumped atoms is Doppler-free in the forward direction with respect to the laser field with a width governed by the homogenous (natural and collisional) lifetime. In the backward direction the radiation is broadened in excess of the Doppler-width. The process operates by compensating for the Doppler velocity-dependent of transition frequencies by shifting and splitting the atomic levels in a balancing velocity-dependent manner by interaction with a saturating laser field on a connecting laser transition sharing a common lower level (Fig. 1).

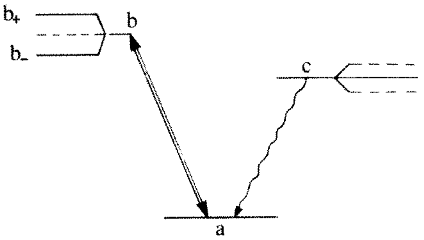

Fig. 1 Energy level scheme for Doppler broadening compensation by light shift perturbations. Levels b and c are Zeeman split by a magnetic field. A circularly polarised laser excites the ab$\pm$ transitions and the linearly-polarised emission from c_0 to a is detected.

Cohen-Tannoudji's first proposal, made at a symposium on frequency standards in 1976, required the use of two saturating laser fields. An optical field detuned by an amount α from a transition frequency of an atom at rest in the laboratory will shift the transition frequency to a new intensity-dependent 'dressed' frequency by a dynamic Stark shift (*Nature* **265,** 683; 1977) which has a dispersion shape. A second laser field detuned an amount α in the opposite direction will produce an equal and opposite Stark shift. The frequency pushing balances the pulling, and for this zero-velocity atom there is no intensity-dependent dynamic Stark shift, or 'light shift'. For an atom with non-zero velocity, then in the atomic rest frame the atom is illuminated by two laser fields with unequal detuning, and the light shifts do not balance out to zero. The laser perturbation of transition frequencies is velocity-dependent, and a careful choice of intensity could counteract the Doppler shift of emission lines.

Together with Hoffbeck and Reynaud, Cohen-Tannoudji (*Opt. Comm.* **27,** 71; 1978) has discussed how the same effect can be produced using one saturating laser field interacting resonantly with two symmetrically detuned atomic transitions separated by a Zeeman splitting (Fig. 1). In Fig. 1, a circularly polarised laser at frequency ω excites the ab_+ and ab_- transitions which have frequencies $\omega+\delta$ and $\omega-\delta$ where δ is the Zeeman splitting caused by a magnetic field. Each of the transitions light-shifts the level a (and b$\pm$). Again for an atom at rest in the laboratory with $v=0$ these light-shifts cancel, but for $v\neq0$ the atomic transition frequencies are intensity-dependent and velocity-dependent in a way that can balance the Doppler shift of emitted lines. For example if the atom sketched in Fig. 1 were weakly excited by a discharge so that radiation were emitted in decaying from another level (way out of resonance with the pump laser on the ab line) c_0 down to a, the emitted light would be Doppler-shifted by a factor $(1-v/c)$ in the forward direction. But if the energy level a were to be light-shifted in a velocity-dependent way as explained, this could shift the ac_0 transition frequency to compensate for the Doppler shift in the forward direction. If this can be arranged for a large enough fraction of the atoms with a spread of velocities, then a significant proportion of the ac_0 transition radiation will be Doppler-free and hence narrowband. Conversely, in the reverse direction the Doppler shift factor is $(1+v/c)$ and the light shift reinforces the Doppler shift, broadening the line to perhaps even double the conventional Doppler width. The special profile of the radiation produced by this 'dressed' three-level system is highly anisotropic. Cohen-Tannoudji *et al.* have determined analytical criteria for Doppler-compensation and discuss the spectra of the emitted light and the way in which the intensity-dependent dressing interactions modify the spectrum of the emitted radiation in the forward direction and produce dynamic-Stark split satellite lines (which they neatly explain in terms of inverse Raman scattering).

Two distinct cases are discussed: partial Doppler compensation for modest laser intensities, where three narrow structures are predicted, and full Doppler compensation for more intense fields where the spectral profile contains a sharp Doppler-free central component and two small Doppler-broadened sidebands. An interesting feature is that the narrowing is entirely due to the energy perturbation of the final state of the transition. The width of the narrow line is governed by parameters such as natural lifetime and collision rates and does not exhibit power-broadening even at high intensities. Other early work on the cancellation of Doppler shifts by a light shift almost linearly dependent on the atomic velocity was reported by Toschek at the 1975 Les Houches meeting (*Frontiers in Laser Spectroscopy* **1,** 329, North Holland, 1977).

Experimental work in progress at ENS using dye laser excitation of ^{20}Ne at 6,163 Å in a magnetic field (to produce the two oppositely detuned Zeeman transitions) and monitoring emission from the third level (c_0 in Fig. 1) has observed the partial Doppler compensation. The emission features in zero magnetic field (a doublet pro-

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duced by the dynamic-Stark splitting of level a by the dye laser field) develops a narrow central feature as the magnetic field is increased, as predicted by Cohen-Tannoudji *et al.* The work has novel implications for Doppler-free superradiance, the observation of Doppler-free coherent transients, and predicts striking forward-backward asymmetries in spectral profiles of great interest in intracavity ring systems. □

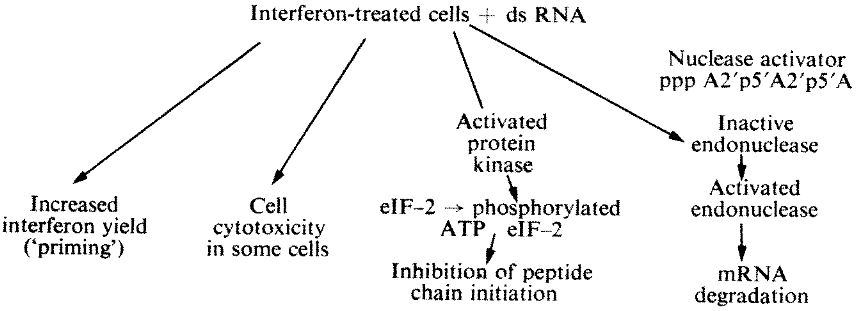
Interferon and double-stranded RNA

from John Morser and Derek Burke

THE *pas de deux* between interferon and double-stranded RNA (dsRNA) continues (see *News and Views* **273,** 97; 1978). It has been known for many years that treatment of cells with double-stranded RNA induces interferon. It now seems likely that viruses also induce interferon by virtue of their ability to make double-stranded RNA, as shown by the elegant study of Marcus and Sekellick (*Nature* **266,** 815; 1977), although additional factors might also be involved (see Kowal & Youngner *Virology* **90,** 90; 1978). When either double-stranded RNAs or viruses are used as inducers, the interferon yield can often be increased by pretreatment of cells with interferon ('priming'). Priming is specific for interferon and it may often turn a non-inducible system into an interferon-producing system. Some cells, notably mouse L cells, also show a pronounced cytotoxicity when treated with interferon and double-stranded RNA (Stewart *et al. Proc. natn. Acad. Sci. U.S.A.* **69,** 1851; 1972). Since such cells also produce interferon, interferon is being synthesised by cells undergoing pronounced cytotoxic damage. Finally, when interferon-treated cells are exposed to double-stranded RNA, translation is blocked, and a series of recent papers (Kerr & Brown *Proc. natn. Acad. Sci. U.S.A.* **75,** 256; 1978; Ball & White *Proc. natn. Acad. Sci. U.S.A.* **75,** 1167; 1978; Lewis *et al. Eur. J. Biochem.* **86,** 497; 1978; Schmidt *et al. FEBS Lett.* **95,** 257; 1978; Farrell *et al. Proc. natn. Acad. Sci. U.S.A.* **75,** 5893; 1978) shows that at least two separate mechanisms are involved. One involves the activation of a protein kinase that phosphorylates, and consequently inactivates the initiation factor eIF-2, and the other involves the formation of pppA2′p5′A2′p5′A ('two-five A') which in turn activates a constitutive inactive endonuclease that degrades mRNA (see diagram).

These effects of interferon require the presence of chromosome 21 in human cells, probably to make the interferon receptor on the cell surface. Both pathways that lead to inhibition of protein synthesis require ATP: the pathway resulting in inhibition of peptide chain initiation for the activation of the protein kinase system, and the one resulting in mRNA degradation to synthesise two-five A by the interferon-induced oligo-isoadenylate synthetase. The scheme reveals a paradox: cells in which protein synthesis has been impaired by two separate mechanisms are also the cells in which interferon mRNA is being actively translated. How can a new protein be formed in such cells? Are any of the intermediates involved in the induction of the interferon gene? The enzyme that synthesises two-five A is possibly one candidate, since it complexes sufficiently well with double-stranded RNA for that to be used as a purification method (Hovanessian *et al. Nature* **268,** 537; 1977), and seems to be present in small amounts in cells which have not yet been exposed to interferon.

One generalisation can be made, however. Interferon sensitises the cell to double-stranded RNA—so that a series of processes which previously occurred either not at all, or at low levels, is now activated. Perhaps this is the control function of interferon—to tell the cells to respond to dsRNA—either added as a synthetic dsRNA (poly (rI)·poly (rC)) or formed as a by-product of virus multiplication.


Interferon-treated cells + ds RNA
Nuclease activator
ppp A2′p5′A2′p5′A
Increased interferon yield ('priming')
Cell cytotoxicity in some cells
Activated protein kinase
eIF-2 → phosphorylated eIF-2
ATP
Inhibition of peptide chain initiation
Inactive endonuclease
Activated endonuclease
mRNA degradation

It is well known that reticulocyte lysates are exquisitely sensitive to the effect of dsRNA, much more sensitive than are cell-free systems made from normal cells and as sensitive as cell-free systems made from interferon-treated cells. Hunt (*News and Views op. cit.*) has suggested that this is because erythrocyte differentiation depends on exposure to interferon at some stage. Certainly interferon antagonises the effect of colony-stimulating factor, inhibits the growth of haemopoetic colony-forming cells and interferes with the normal differentiation of granulocyte–macrophage precursors (McNeil & Fleming in *The Interferon System: a Current Review to 1978,* Texas Reports on Biology & Medicine (eds Baron & Dianzani) 343; 1978). Interferon also inhibits the expression of erythroid differentiation in Friend leukaemia cells treated with dimethylsulphoxide (Rossi *et al. ibid* page 420).

If interferon is involved in any way in differentiation, is this the reason why the interferon system is an inducible rather than a constitutive system and why it is completely uninducible in mouse teratocarcinoma stem cells (Burke *et al. Cell* **13,** 243; 1978)? Perhaps dsRNA is a pleiotropic effector, but only active in interferon-treated cells? We do not yet know the answers to these questions, but perhaps we should look harder for double-stranded RNA elsewhere. □

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Radioactive waste management

from S. Weisenburger

THIS interdisciplinary meeting*, covering a broad range of topics, from the processing, solidification, and characterisation to the isolation of radioactive wastes arising from the nuclear fuel cycle was devoted especially to scientific research aspects of radioactive waste management. These aspects have often been relegated to the sidelines in international gatherings chiefly concerned with systems management.

A general review on the management of nuclear waste by Rustum Roy

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*An International Symposium on 'Science underlying waste management' was held in Boston on 28 November–1 December, 1978 and sponsored by the Materials Research Society. The proceedings will be available in Spring, 1979.

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(Pennsylvania State University) ranged from the selection of suitable matrix materials for different types of nuclear wastes to geological considerations relevant to nuclear waste storage.

Several schemes of radioactive waste solidification are currently under consideration. Most papers dealt with matrix materials for high-level radioactive waste (HLW). The technologically most advanced solidification process for HLW is vitrification, which is already in use (for example the French AVM process). However, there are still very intensive research and development efforts aimed at putting the waste into solid forms other than glass, thus providing even better barriers.

Processes based on multibarrier encapsulation concepts were also presented. An alternative approach is to incorporate the radionuclides into minerals (or a synthetic host simulating a mineral) known to have been stable for millions of years in the past. The fixation of HLW in cermet form with an iron-nickel base was also considered. Multibarrier encapsulation relies on up to three barriers to isolate radionuclides from the environment: a solid waste inner core, an impervious coating and a metal matrix. Two candidate materials for the inner core have been suggested: sintered ceramic and glass marbles. If it were desirable to produce a waste form with enhanced inertnes (compared to monolithic glass) but with minimum technological complexity, then glass marbles encapsulated in a vacuum-cast lead alloy was recommended for further development.

An example of incorporating HLW into minerals is the SYNROC-process which has been developed on a laboratory scale. SYNROC is a synthetic rock composed of phases such as hollandite, cubic zirconia, perovskite and celsian and its bulk composition is characterised by a high content of TiO_2 (35%) and a low content of SiO_2 (12%). Calcined HLW can be added to SYNROC-melts and the mineral assemblage can be formed by direct crystallisation from a melt cooled below 1300 °C, or by hot pressing under subsolidus conditions at 1100–1250 °C. The product is considered to be highly resistant to alteration and leaching in any geological environment. The SYNROC process is far from ready to be put into practice in an engineering sense and some important aspects are not entirely clear.

Many papers dealt with the characteristics of nuclear waste in forms including glass, glass-ceramic, ceramic, minerals, cement, and concrete. Besides microstructural characterisation, the measurements of leach rates and leach mechanism of solid radioactive waste forms were of major concern. Detailed knowledge about leaching is required to establish which are the hazardous radionuclides if and when the waste comes into contact with a leaching medium. The specific radionuclides of concern are relatively few (^{137}Cs, ^{90}Sr, rare earths, actinides). It was shown that the leaching process can be highly complex and that the chemistry of nuclear waste glass reactions must take into account a multitude of diffusing species, as well as changes in the leach interface, and that the solvent chemistry may not remain constant throughout a test run. Expressions were given which fit the leach data and which were based on diffusion models in combination with other influences (time-dependent surface concentration of mobile species, dissolution and precipitation of mobile species, dissolution of matrix material). A relatively new method was described which allows measurements of compositional profiles in the leached waste material using ion beam spectrochemical analysis.

The main subject of the presentations dealing with radioactive waste in geological settings was the detailed analysis of the interaction of waste glass with the geological environment. The most credible scenario for rapid mobilisation of the radionuclides contained in the waste is dissolution due to mobility of indigenous water in the host rock or intrusion of ground water in the repository. The combination of radioactive decay heat (in the first few hundred years) and pressure would then create a 'hydrothermal' environment (up to 400 °C, 300 bar) that may accelerate corrosion and breaching of canisters. Results of experimental work show that the hydrothermally reacted glass forms complex produces. Under the applied experimental conditions the glass tends to convert to a substantial extent into crystalline phases (50%), the major component being $NaFeSi_2O_6$. Bulk X-ray fluorescence analysis of the surface of the reacted glass had shown that Cs, Rb, and Mo were significantly leached into the aqueous phase. Methods were proposed to protect reactive waste from hydrothermal conditions or to produce waste forms which can withstand such conditions (multibarrier waste isolation shows significant promise).

Thirteen papers dealt with radionuclide transport phenomena in geomedia. There is still a paucity of quantitative estimates of the various geological materials' ability to retard nuclide movement, but progress does appear to have been made in establishing a data bank for sorption and migration of radionuclides in different geological media. These data are urgently needed as part of the input for modelling and safety assessment of geological waste repositories. A number of thermodynamic as well as kinetic parameters influence sorption and migration data and it is now being realised that the numerous experimental designs used for their measurement should be replaced by standard techniques. Any prediction of the long-term sorption and migration behaviour of the radionuclides, must take into account the effects of specific variables such as permeability, porosity, cation exchange capacity, particle size and surface area.

Modelling and safety assessment is mainly concerned with detailed analyses of the full spectrum of phenomena which alone or in consort, could conceivably lead to significant leaching. Results were presented from a pilot computer program based on a model for a geological system containing a waste repository.

To summarise, the symposium showed that extensive scientific research is being done in the field of nuclear waste management. However, it is also clear that much more research and development is required before the behaviour of radioactive waste is fully understood. □

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NATURE

A hundred years ago

WE learn from the *Journal de St. Petersburg* that the epidemic in Astrakan was discussed before the Russian Medical Society at a gathering where 800 were present. It seems that the people call it the plague, though it is not officially so known. M. Botkine mentioned that at the time of the last plague at Moscow in 1770, the question was discussed whether it was the true plague or a marked form of typhus, and he added that the diagnosis of the various forms of typhoid infection in Russia is very difficult. He believes that the spots on the body and the quickness with which death follows indicate that the present epidemic of Vetlianka is not a European malady. Dr. Nicolaïew, describing the symptomatology of the plague, said that its action is both physical and moral, and that to impose quarantine often helps rather than retards the spread of the disease by the fear it awakens.

From *Nature* **19** 6 February, 325; 1879.

articles

Measurements of general relativistic effects in the binary pulsar PSR1913+16

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Measurements of second- and third-order relativistic effects in the orbit of binary pulsar PSR1913+16 have yielded self-consistent estimates of the masses of the pulsar and its companion, quantitative confirmation of the existence of gravitational radiation at the level predicted by general relativity, and detection of geodetic precession of the pulsar spin axis.

THE earliest observations of binary pulsar PSR1913+16 showed that, because its orbit involved large velocities ($v/c \approx 10^{-3}$), a high eccentricity ($e \approx 0.617$), and relatively strong gravitational fields ($GM/c^2r \approx 10^{-6}$), several special and general relativistic effects should eventually be observable[1]. The advance of periastron (at the rate $\dot{\omega} \approx 4.2$ deg yr^{-1}) was the first of these effects to be measured[2], and the rate of advance has now been determined to better than 0.1% accuracy[3,4]. We report here the detection of four more effects of relativistic origin, including quantitative measurements of three of them. Together with the much larger effects already measured, the new parameters over-determine the system and provide: (1) the first determination of the mass of a radio frequency pulsar; (2) constraints on the nature of the companion star, and a measurement of its mass; (3) determination of the angle of inclination between the plane of the orbit and the plane of the sky; (4) quantitative confirmation of the existence of gravitational radiation at the level predicted by general relativity; and (5) qualitative observation of geodetic precession of the pulsar spin axis. The data are consistent with the general theory of relativity and provide some strong constraints on any alternative theory of gravitation.

Pulse arrival-time data

Most of the new information comes as the result of pulse arrival-time measurements, which now comprise approximately 1,000 observations spanning 4.1 yr. The data were obtained at frequencies near 430 and 1,410 MHz, using the 305-m radio telescope at Arecibo, Puerto Rico. The normal observing procedure involves adding together approximately 5,000 pulses, using a pre-computed ephemeris to define the expected pulsation period. The resulting mean pulse profile is then fitted by the method of least squares to a long-term average 'standard profile', to obtain the precise pulse arrival time. Because of improvements in equipment and techniques between 1974 and 1978, our timing accuracy has gradually improved[3-5] from ~1 ms to ~50 μs. Data have been acquired at intervals not exceeding 7 months, and in spite of the short period of the pulsar ($P = 0.059$ s), there has been no problem in keeping track of the number of elapsed pulse periods.

Our analysis of the timing data follows the formulation of Epstein[6], and proceeds by the following steps. First, the pulse arrival times are corrected from the location of the observatory to the barycentre of the Solar System, including a relativistic clock correction to account for annual changes in gravitational potential at the Earth. A correction is then made for the dispersive delay in the interstellar medium, using the frequency of observation as Doppler-shifted by the Earth's motion. Finally, the proper time t_p in the pulsar's reference frame is obtained by correcting for (1) the projection onto the line of sight of the pulsar's orbital position, (2) the integrated effects of gravitational redshift and transverse Doppler shift in the highly eccentric pulsar orbit, and (3) the gravitational propagation delay. These three corrections have magnitudes of order (v/c), $(v/c)^2$ and $(v/c)^3$ times the orbital period, respectively. On the basis of initial guesses for the various parameters that enter the timing equation (see Table 1), the pulsar phrase at the time of observation can be predicted from the relationship

$$\phi = \phi_0 + t_p/P - \dot{P}t_p^2/(2P^2) \qquad (1)$$

This phase should be very close to an integer; the difference, or phase residual in periods, is used in a least-squares solution for improved values of the model parameters.

Table 1 Parameters derived from timing data

Right ascension (1950.0)	$\alpha = 19$ h 13 min 12.474 s ± 0.004 s
Declination (1950.0)	$\delta = 16° 01' 08''.02 \pm 0''.06$
Period	$P = 0.059029995269 \pm 2$ s
Derivative of period	$\dot{P} = (8.64 \pm 0.02) \times 10^{-18}$ s s^{-1}
Projected semimajor axis	$a_1 \sin i = 2.3424 \pm 0.0007$ light s
Orbital eccentricity	$e = 0.617155 \pm 0.000007$
Binary orbit period	$P_b = 27906.98172 \pm 0.00005$ s
Longitude of periastron	$\omega_0 = 178.864 \pm 0.002°$
Time of periastron passage	$T_0 =$ JD 2442321.433206 ± 0.000001
Rate of advance of periastron	$\dot{\omega} = 4.226 \pm 0.002$ deg yr^{-1}
Transverse Doppler and gravitational redshift	$\gamma = 0.0047 \pm 0.0007$ s
Sine of inclination angle	$\sin i = 0.81 \pm 0.16$
Derivative of orbit period	$\dot{P}_b = (-3.2 \pm 0.6) \times 10^{-12}$ s s^{-1}

Quoted uncertainties are twice the formal standard errors from the least-squares fit.

As indicated in Table 1, our timing model contains 13 parameters of physical interest, all of which must be determined

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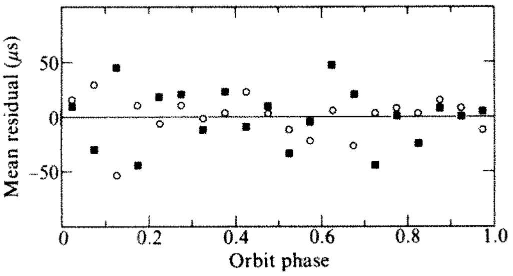

Fig. 1 Differences between observed and computed pulse arrival times for PSR1913+16, after averaging the data into 20 equal intervals of orbit phase for each of two observing frequencies: ■, 430 MHz; ○, 1,410 MHz. Parameter values listed in Table 1 were assumed for the computations, together with a dispersion measure of 171.64 cm^{-3} pc.

from the timing data. The parameters include the celestial coordinates of the system α and δ; the period and period derivative of the pular 'clock', P and $\dot{P}$; five Keplerian orbit parameters; and four parameters which measure effects of special and general relativistic origin. In addition to the advance of periastron, the relativistic terms include γ, which measures the variable part of the time delays contributed by transverse Doppler shift and gravitational redshift; sin i, the sine of the angle of inclination between the orbit and the plane of the sky, which is measurable because of its influence on both the gravitational propagation delay and the post-newtonian corrections to the elliptical orbit; and $\dot{P}_b$, the rate of change of orbital period. Further details concerning the timing model and methods of treating the data may be found elsewhere[3,6–8].

Parameter values based on data acquired through October 1978 are listed in Table 1. The weighted root mean square deviation of the 837 arrival-time measurements about the fitting function is 80 μs. In an attempt to search for possible low-level systematic departures of the data from the model, the residuals were averaged in 20 equally spaced segments of orbit phase. The resulting phase-averaged residuals are plotted in Fig. 1, with 430 MHz data and 1,410 MHz data shown separately. The points seem to be scattered at random about the zero line, with no phase dependent errors exceeding ~20 μs. We conclude that the timing model being used provides an exact fit to the data, within our measurement uncertainties, and therefore that no significant effects have been omitted from the analysis.

Except for $\dot{\omega}$, the relativistic parameters have not previously been measurable to useful accuracies. As shown in Table 1, all of them have now been determined to within ⩽20% accuracy. The most uncertain of the four quantities is sin i, because the terms in the timing equation on which it depends have the smallest amplitude[6,7], of order $P_b(v/c)^3 \approx 30$ μs. Because this amplitude is somewhat smaller than the typical individual measurement uncertainties, we performed an F-test for the significance of including sin i in the fit. The test gives a positive result at the 95% confidence level, and thus we believe that the value quoted for sin i is valid.

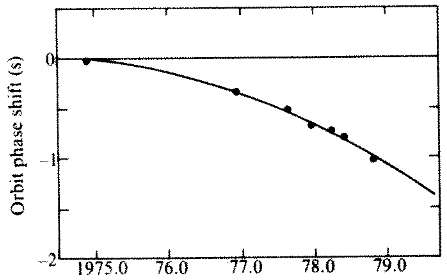

Fig. 2 Curves to delimit possible masses of the pulsar and its companion, based on the observed values of γ, sin i and $\dot{P}_b$. On these criteria, the most probable masses lie in the shaded region. The sloping straight line is the locus of all mass pairs for which the general relativistic contribution to $\dot{\omega}$ is the observed value, 4.266 deg yr^{-1}. The emphasised portion of this line is consistent with the measured values of γ and sin i and very close to the measured value of $\dot{P}_b$; it probably represents the best available estimates of the masses.

Together with the well determined Keplerian parameters, the values of γ, sin i and $\dot{P}_b$ provide strong constraints on the masses of the pulsar and its companion (m_p and m_c, respectively). These constraints can be illustrated conveniently in a diagram of pulsar mass against companion mass, as shown in Fig. 2. In constructing this figure we have used the relationships

$$\gamma = 0.002951\left(\frac{m_c}{M_\odot}\right)\left(\frac{m_p+2m_c}{M_\odot}\right)\left(\frac{m_p+m_c}{M_\odot}\right)^{-4/3} \text{ s} \qquad (2)$$

$$\sin i = 0.5083\left(\frac{m_c}{M_\odot}\right)^{-1}\left(\frac{m_p+m_c}{M_\odot}\right)^{2/3} \qquad (3)$$

$$\dot{P}_b = -1.70\times10^{-12}\,\frac{m_p m_c}{M_\odot^2}\left(\frac{m_p+m_c}{M_\odot}\right)^{-1/3} \text{ s s}^{-1} \qquad (4)$$

$$\dot{\omega}_{GR} = 2.11\left(\frac{m_p+m_c}{M_\odot}\right)^{2/3} \text{ deg yr}^{-1} \qquad (5)$$

which follow directly from equations summarised in ref. 3 and the parameters given in Table 1. Curves have been plotted in Fig. 2 bracketing the range of values of γ, sin i and $\dot{P}_b$ consistent with the data; they suggest a combination of pulsar mass and companion mass somewhere within the shaded region, that is $m_p \approx m_c \approx 1.6\,M_\odot$. According to equation (5), one would then expect to observe $\dot{\omega} = 4.58$ deg yr^{-1}. Because the observed value of $\dot{\omega}$ is well determined at ~10% less than this value, it seems that a much more probable solution for the masses lies somewhere along the emphasised portion of the sloping straight line, such that $m_p = 1.39 \pm 0.15\,M_\odot$, $m_c = 1.44 \pm 0.15\,M_\odot$, and that the observed rate of periastron advance is entirely caused by the general relativistic effect. In this case, the observed magnitude of $\dot{P}_b$ agrees with the value expected from gravitational radiation damping to within a factor 1.3±0.3. There is no compelling evidence for significant tidal or rotationally-induced contributions to $\dot{\omega}$.

Undoubtedly the most important new result of this work is the measurement of $\dot{P}_b$ with the sign and magnitude expected on the basis of gravitational radiation within general relativity. The validity of the measurement is clearly shown in Fig. 3, which shows the accumulating orbit phase error caused by assuming P_b fixed at its 1974.9 value, that is $\dot{P}_b = 0$. The measured points (which correspond to separate determinations of the time of periastron passage, T_0, for each of seven major observing sessions) are not well fit by a straight line. However, they fall very close to the plotted parabola, which represents the general relativistic prediction for $m_p = m_c = 1.41\,M_\odot$ according to equation (4). The data thus provide a striking confirmation of a longstanding prediction of the general theory of relativity, and an indirect proof of the existence of gravitational waves carrying energy away from the orbiting system.

Other possible contributions to $\dot{P}_b$ seem to be implausible, *ad hoc*, or of negligible magnitude. For example, differential galactic rotation and mass loss from the system contribute at most about ~1% (refs 9, 16, 21) of the observed value of $\dot{P}_b$. If the (as yet unidentified) companion object is a neutron star or a black hole, then tidal dissipation is also of no significance. Tidal effects could be important in a white dwarf companion, but the only white dwarfs consistent with $\dot{\omega} = 4.266$ deg yr^{-1} have either rapid rotation[9] or masses close to the Chandrasekhar limit. Such stars would produce either positive or negligible contributions[9,22,23] to $\dot{P}_b$. We conclude that the only straightforward interpretation of the observed orbital decay is in terms of gravitational radiation damping.

Gravitational radiation

It has already been pointed out[10,11] that most theories of gravity other than general relativity predict dipole gravitational radiation from an orbiting system like PSR1913+16. In these theories, the predicted dipole radiation is considerably larger than the quadrupole radiation in general relativity, and in some theories it even carries energy of the opposite sign[11]. Unless the two orbiting stars are within a few per cent of being identical in their ratios of gravitational binding energy to inertial mass (in which case the dipole radiation term vanishes) it is difficult to reconcile our measurement of $\dot{P}_b$ with any such theory. The data are also inconsistent with the suggestion that gravitational waves may be composed of half-retarded, half-advanced potentials, and would therefore carry no energy at all[12].

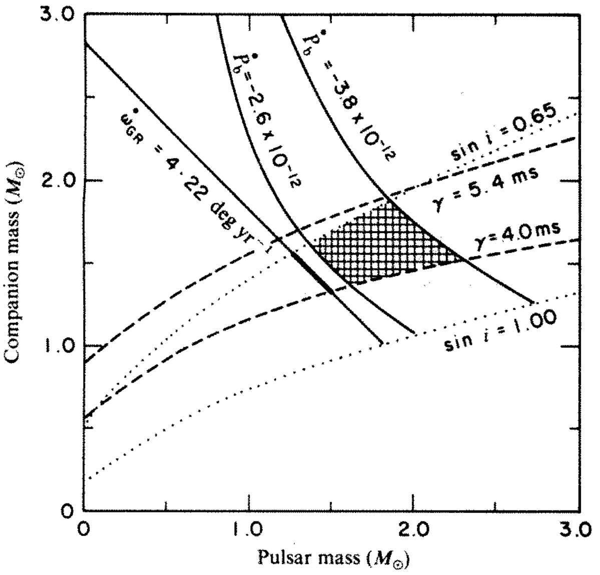

Fig. 3 The points represent measured orbital phase errors caused by assuming a fixed value of P_b. Uncertainties associated with each point are comparable with or smaller than the point itself. The plotted curve corresponds to the orbital period derivative predicted by general relativity if $m_p = m_c = 1.41\ M_\odot$.

An argument has been put forth[13,14] that a formula for energy loss due to gravitational radiation has not yet been derived consistently within general relativity, and that the quadrupole formula used[15,16] to derive equation (4) may be inaccurate because it ignores nonlinearities caused by radiation-reaction forces. As shown in Fig. 2, our data suggest that any such inaccuracy is not very large. The uncertainty associated with $\dot{P}_b$ will decrease rapidly with continued measurements over the next few years, so it will soon become evident if there is any significance to the small apparent discrepancy.

The new mass determinations are consistent with current ideas about neutron star structure and formation and with the hypothesis, based on evolutionary grounds, that the binary system consists of two neutron stars[9,17,18]. A main sequence helium star[19] can now be ruled out as a companion, because it would require $\dot{P}_b \approx -0.5 \times 10^{-12}$, about one-sixth of the observed value.

General relativity also predicts[15,20,24] that in this system geodetic spin–orbit coupling should cause the pulsar spin axis to precess at a rate $\Omega_p \approx \frac{7}{24}\dot{\omega}_{GR} \approx 1$ deg yr^{-1}. According to currently viable pulsar theories, a double-peaked pulse shape such as that observed for PSR1913+16 is probably caused by the rotation through our line of sight of a hollow cone of beamed emission. Thus, as the spin axis precesses, we should expect to observe different cuts through the radiation beam, probably accompanied by changes in pulse width and/or shape.

Such an effect has now been observed, as shown in Fig. 4. Here we plot the average pulse profiles observed in July 1977, June 1978 and October 1978, with the 1978 data smoothed so as to have the same time resolution (400 μs) as the earlier profile. The three profiles have been aligned at the leading edge and normalised so that the first component has identical height in all three cases. With this convention, the leading component does not seem to have changed significantly, but the central component has broadened substantially and moved towards the pulse centre. At the same time the separation between outermost peaks has progressively increased, and the third component has also broadened somewhat. The extreme trailing edge of the pulse seems to fall more rapidly in the October 1978 profile than in the earlier ones. However, this trailing edge is considerably wider than that observed[4] at 1,400 MHz, probably because of interstellar scattering. Consequently, this part of the pulse shape change may result from a change in the scattering time constant of the interstellar medium.

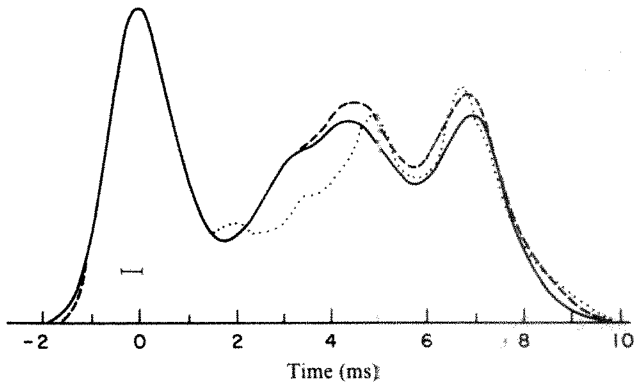

Fig. 4 Pulse profiles observed at 430 MHz in July 1977 (dotted line) and June (dashed line) and October (solid line) 1978. The central component has been gradually moving to the left and becoming broader, while the third component has shifted to the right. All profiles have been smoothed to the resolution indicated by the horizontal bar, 400 μs.

Changes of pulse shape towards a broader and more symmetrical profile suggest that precession is moving the observer's line of sight closer to the centre of the radiation cone. A geometry that accounts qualitatively for the observed changes is shown schematically in Fig. 5. More data will be required over a longer time interval, preferably including polarisation

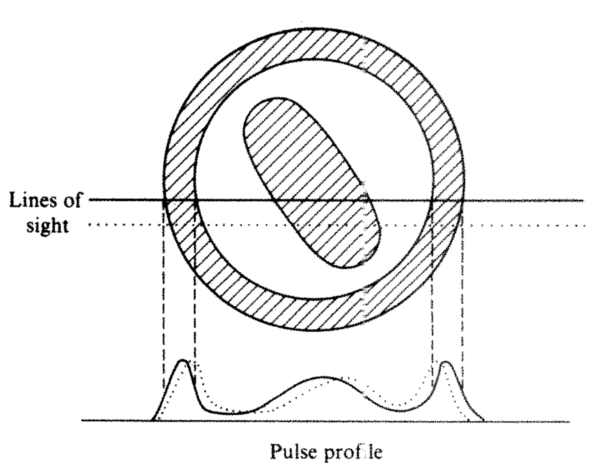

Fig. 5 A possible geometry to account for the pulse shape changes shown in Fig. 4. Shaded regions represent active portions of a hollow cone of pulsar emission; horizontal lines represent loci of the line of sight through the beam as the pulsar spins. Hypothetical pulse profiles are sketched below. As the spin axis precesses, moving a different portion of the beam into the line ofvt, the profile is expected to change. October 1978 (solid line); July 1977 (dotted line).

information, before any quantitative description of the observed changes of angle can be made.

Our measurement of the rate of change of orbital period is an entirely new test of general relativity, and at present the only one that depends on the theory's structure beyond the first post-newtonian approximation. The only straightforward interpretation of the data is that gravitational waves exist and carry energy away from an orbiting system at the rate predicted by general relativity. The conclusions that $m_p \approx m_c \approx 1.4\ M_\odot$ and $\dot{\omega} \approx \dot{\omega}_{GR}$ give strong support to the interpretation of the PSR1913+16 system as a pair of neutron stars, and provide an important test of the theory of late stages of stellar evolution. Finally, the observed precession of the pulsar spin axis provides a unique opportunity to obtain information on the shape of the pulsar radiation beam in two dimensions.

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Vulcanian eruption mechanisms

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Application of a newly developed theory to volcanological observations of explosive eruptions shows that previous estimates of pre-explosion gas pressures may be over-estimated by an order of magnitude.

THE andesite stratovolcano Ngauruhoe, in North Island New Zealand, erupted explosively during the afternoon of 19 February 1975. The eruption was closely observed and filmed[1]. There were two main phases. A 1.5-h gas-streaming phase[2] produced an 11–13 km high eruption column. This phase was followed by a series of discrete cannon-like explosions at 15 to 50 min intervals over a period of 5 h (ref. 2). The latter type of activity is common on many andesite and basaltic andesite volcanoes, for example Asama[3] and Arenal[4], and is referred to as vulcanian type activity[5,6].

Vulcanian explosions have been studied from several recent eruptions[7,8] and display common characteristics: the rock mass ejected per explosion is usually 10^2 to 10^6 tonnes (refs 3, 6) and often contains a high proportion ($\geqslant$50%) of nonjuvenile material; during active periods, intervals between explosions vary from <1 min (Anak Krakatoa[6]) to about 1 day (Asama[3], Sakurazima[9]); pyroclastic avalanches (*nuées ardentes*) are often produced[8,10,11].

Nairn and Self[2] recently described the February 1975 eruption of Ngauruhoe and interpreted the generation of cannon-like explosions in the light of concepts established in the volcanological literature. Here we use newly developed theory to re-analyse the largest of the cannon-like explosions (at 18.10 h on 19 February) and discuss an alternative model of vulcanian explosions. The model is constrained by paucity of data, particularly on lava tensile strengths and weight percentages of fine ejecta and released gases, but does point out the type of data required for improved analysis of vulcanian eruptions. McBirney[12] recently reviewed controls on explosive andesitic eruptions and criticised the use of the Bernouilli equation to derive pre-explosion pressures. However, use of the 'gun-barrel' equation, recommended by McBirney, is also open to criticism. This article revises some earlier computations[2] of pre-explosion pressures at Ngauruhoe and demonstrates that gas pressures required to generate vulcanian explosions are probably lower than previously thought.

Direct observations of discrete vulcanian explosions

The best documented discrete explosion at 18.10 h consisted of the expulsion of a cloud of gas containing fragments with a wide range of sizes. The initial velocity was supersonic, resulting in a shock wave[2]. The position and extent of the cloud was recorded on a series of photographs timed with sufficient accuracy for estimates to be made of the average velocity between each pair of photographs. Figure 1 shows the resulting variation of velocity with height; an estimate of errors has been included.

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It is known that the velocity of a cloud of material ejected into the atmosphere at high speed must decrease approximately exponentially with height[13] after passing through a maximum value, thus one estimate of the initial velocity can be obtained by fitting a straight line to the data shown in Fig. 1: the value indicated is ~400 m s^{-1}. A second estimate comes from consideration of the dynamics of the shock wave propagation: to produce the apparent shock velocity of at least 600 m s^{-1} the velocity of the ejected slug of debris would have to be not less than 340 m s^{-1} (ref. 2) calculated for a one-dimensional case. The true maximum velocity of the ejecta probably lies between these values.

Theory of vulcanian explosions

Discrete, intermittent explosions, with the exception of phreatomagmatic explosions, must be the result of the sudden release of pressure in a gas by the failure of some form of retaining medium. In the vulcanian case the gas may be magmatic or meteoric. It seems improbable that a layer of unconsolidated clasts at the top of the conduit can substantially retain the build-up of an appreciable gas pressure. We assume, therefore, that magma is always involved in such events. New magma rises in a conduit after a previous explosion and cools at its upper surface, forming a 'cap'.

The compressional strength of dolerite is known to remain nearly constant[14] with temperature up to 950 °C; in view of the lack of evidence to the contrary, we assume that tensile strengths of rocks, commonly quoted to be of the order of 10% of compressional strengths, also remain constant up to this temperature. Tensile strengths (at low pressures) of 100–200 bar are deduced for rocks of andesitic type from the limited data available[14,15]. To obtain an estimate of the thickness of crust sufficiently cool to provide a strength of this order, standard treatments on heat flow can be used[16]. Cooling from magmatic temperature (say 1,000 °C) during the 50 min interval before the 18.10 h explosion could have produced a layer, cooler than 950 °C, which was up to 15 cm thick, the distance a thermal wave can travel in rock in this time. Clearly, cool fragments of rock debris smaller than 30 cm in diameter would be efficient heat sinks on this time scale. Indeed, the addition of 10% by weight of such debris, derived from the conduit walls or the fall-back in the vent area from earlier explosions, would cool a layer of any desired thickness to 950 °C.

The pressure in a mixture of degassing magma and heated groundwater prior to an explosion would be equal to the tensile strength at depth H below the cap by an amount $\sigma_r gH$ where σ_r is the magma density and g the acceleration due to gravity. At a depth of 500 m in partially degassed magma of bulk density 1,000 kg m^{-3} the added pressure would be about 50 bar.

Many attempts have been made to relate the ejection velocity of fragments from volcanic explosions, particularly those of vulcanian type, to the initial pressure of compressed gas driving the explosion[6,8,12,17,18]. All of these treatments are essentially based on a modified form of the Bernouilli equation. We are no longer satisfied that this equation adequately represents the conditions in any actual volcanic explosion.

The simplest treatment of an explosion driven by expanding gas is one in which it is assumed that complete decompression to atmospheric pressure occurs, and that all of the internal energy released by the expansion is used to accelerate the explosion products, both released gas and clasts, to the same velocity. The energy equation[19] is then:

$$-\int \frac{\mathrm{d}P}{\rho} = \int g\,\mathrm{d}h + \int u\,\mathrm{d}u \qquad (1)$$

in which ρ is the bulk density of the eruption products, P is the pressure, g is the acceleration due to gravity, h is the vertical coordinate and u the velocity. ρ must be defined in terms of n, the weight fraction of released gas in the exploding mixture, σ, the gas density, and σ_r, the clast density:

$$\frac{1}{\rho} = \frac{n}{\sigma} + \frac{1-n}{\sigma_r} \qquad (2)$$

σ is in turn dictated by the temperature, pressure and composition of the gas; in the present circumstances it is adequate to assume that the perfect gas law applies. It is necessary, before integrating equation (1), to decide whether the gas expansion will be more nearly isothermal or adiabatic; the deciding factor is the efficiency with which heat can be transferred from clasts to gas and depends on the clast size distribution[20]. In the case of strombolian explosions, in which the time scale is very short, an adiabatic approximation is valid[13]. For vulcanian explosions, the best solution probably lies between the isothermal and adiabatic cases. The results of integrating (1) using (2) and an initial temperature T_i are:

$$nRT_i \ln (P_i/P_f) + \frac{(1-n)}{\sigma_r}(P_i - P_f) = g\Delta h + \tfrac{1}{2}u_f^2 \qquad (3)$$

in the isothermal case and, in the adiabatic case:

$$nRT_i \frac{\gamma}{(\gamma-1)}\left[1-\left(\frac{P_f}{P_i}\right)^{(\gamma-1)/1}\right] + \frac{(1-n)}{\sigma_r}(P_i - P_f) = g\Delta h + \tfrac{1}{2}u_f^2 \qquad (4)$$

where P_i is the initial pressure driving the explosion, P_f is the final pressure, assumed equal to atmospheric, Δh is the vertical distance over which the gas decompression occurs, γ is the ratio of the specific heats of the gas, R is the gas constant and u_f is the final velocity. If numerical values are inserted, it is generally found that the term $g\Delta h$ can be neglected for explosions in which the expansion of gas occurs over a vertical distance of less than a few hundred metres.

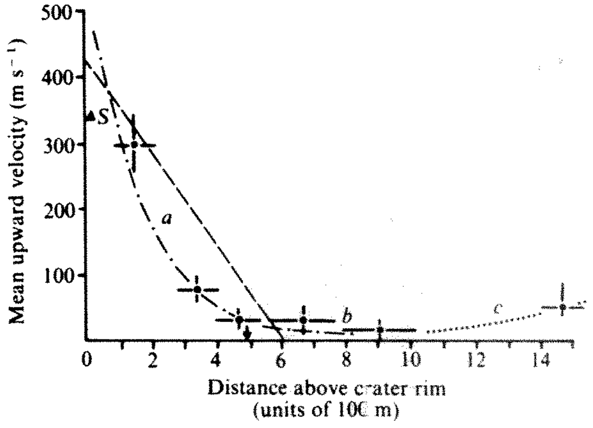

Fig. 1 Deceleration of 18.10 h plume from analysis of still photographs. Errors shown as bars. Straight line fitted between maximum and minimum values; curved line approximates exponential deceleration. S is speed of sound in air for reference. The steep portion of curve (a) represents deceleration in gas thrust phase[7]; the flat portion (b) represents slow, stable velocity condition while mixing with air and column collapse takes place; (c) is a slight increase in velocity at beginning of convective thrust phase. The downwards pointing arrow indicates onset of column collapse.

The modified version of the Bernouilli equation is usually written:

$$P_i - P_f = \tfrac{1}{2}\sigma_r u_f^2 \qquad (5)$$

in terms of the variables already defined. There has been some discussion[12] as to what density should appear in this equation: σ_r (as written here) or ρ. Comparison of equations (5) and (3) reveals the essential problem with the modified Bernouilli equation: it only approximates the correct expression if n is sufficiently small (and if Δh is also small). The values of n encountered in real volcanic explosions (say 0.1 to 30 weight per cent if plinian, vulcanian and strombolian events are included) are always too large for this approximation to be true.

We have argued above that the build-up of pressure beneath a restraining cap may be the source of vulcanian explosions. In such a case the mixing of gas and clasts from the disrupted cap and the surrounding rocks may not be great in the early part of the motion; a physical model based on the motion of a solid block of coherent (or shattered) rock overlying a gas body of similar cross sectional area may be more applicable. If the vertical extent of the cap is L and its density is σ_r, and if the gas body beneath the cap has vertical extent X, initial temperature T_i, pressure P_i and gas constant R, then simple geometry shows that

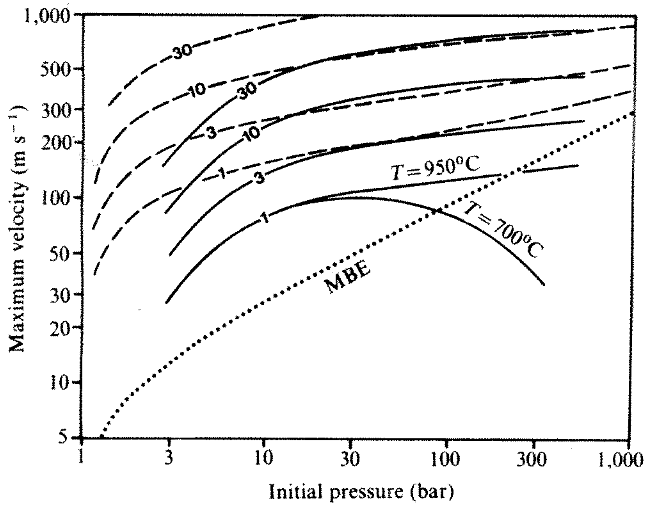

Fig. 2 Maximum velocity of explosion products as a function of initial pressure driving the gas expansion for various types of volcanic explosions. The dotted line MBE is the modified Bernouilli equation (equation (5)). The dashed lines are calculations using equation (3) and are labelled by the wt % gas (taken to be steam) used in the calculations. For these curves perfect thermal contact between gas and clasts is assumed. The solid curves, also labelled by wt % gas in the exploding mixture, represent solutions to equation (7); the gas cools adiabatically during its expansion from an initial temperature of 1,220 K; in the case of the curve for 1% gas, the effect of reducing the initial temperature by 250 degrees is shown. The dashed and continuous curve for each gas content bracket the upper and lower limits of velocity for a given driving pressure in vulcanian explosions. When the ejecta are predominantly less than 10 mm in diameter, velocity values correspond more closely to the dashed curves.

$$\frac{X}{L}=\left(\frac{n}{1-n}\right)\left(\frac{\sigma_r R T_i}{P_i}\right) \tag{6}$$

so that the solutions may again be expressed in terms of n, the gas weight fraction in the explosion products. The equation of motion for the cap in the vertical h direction is

$$P-P_f=L\sigma_r\ddot{h}+L\sigma_r g+\tfrac{1}{2}\rho_a C_D\dot{h}^2 \tag{7}$$

where P is the general value of the pressure during the expansion. P is given by

$$P=P_i\left(\frac{X}{X+h}\right)^{\gamma} \tag{8}$$

initially, since the expansion is adiabatic until some small clasts begin to mix with, and supply heat to, the gas. The final term in equation (7) represents atmospheric drag acting on the rising cap: C_D is a drag coefficient with value close to unity and ρ_a is the atmospheric density. Equation (7) is readily integrated numerically using equations (6) and (8) to yield the maximum velocity, u_f, of the products during the explosion process. The results of such calculations, and also of the use of the simpler equations (3) and (4), are summarised in Fig. 2, in which u_f is shown as a function of P_i for a range of values of n in each case.

It is likely that at least 50 wt % of the fragments ejected during the 18.10 h explosion were smaller than 3 mm (ref. 2) and able, therefore, to maintain thermal equilibrium with the gas during the period (of the order of 0.5–2 s) of acceleration of the largest clasts, implies that the velocities corresponding to equation (3) (isothermal case) may be more appropriate for vulcanian explosions than those derived from equation (4); the solutions to equation (7) certainly represent the lowest possible velocity that corresponds to any particular pair of values of n and P, as they give the greatest weight to energy losses. The speeds given by equation (4) are very close to those from equation (3) at low pressures and fall to about 70% of the equation (3) speeds as the pressure approaches 1,000 bar. The n and P values given below are means of equations (3) and (7) biased towards equation (3) on the basis of this argument.

The following combinations of initial gas pressure and released gas wt % are implied by the observed maximum velocity of 400 m s^{-1}: for $n=30\%$, $P_i=2$–5 bar; for $n=10\%$, $P_i=10$–30 bar; for $n=5\%$, $P_i=40$–100 bar; for $n=3\%$, $P_i=100$–300 bar. We argued above that the likely range of values of tensile strength of andesite at sub-solidus temperatures is 100–200 bar (refs 14, 15). Then the above permutations of pressure and gas content would imply that the explosion products of the 18.10 h event contained 2–5 wt % water. It was estimated[2] that equal proportions of juvenile and nonjuvenile material were expelled in the explosion, and so if it is assumed that all of the water were juvenile, 4 to 10 wt % would be required in the magma. The lower end of this range is comparable to the measured primary water contents[21,22,28] of andesite magmas, but it is probably implied that some groundwater water was also involved.

Magma volume and conduit shape considerations

The total volume of rock expelled in the 18.10 h explosion and four other similar outbursts on the same day was about 5×10^5 m^3 dense rock, approximately half of which was juvenile. We may assume that juvenile material was re-emplaced in the upper parts of the volcano between explosions, so that a total of about 2.5×10^5 m^3 of pre-existing cone material was excavated.

This volume cannot have been removed from a localised region near the vent because we know[2] that there was little significant change in the geometry of this region during the explosive activity: if 2.5×10^5 m^3 is taken to occupy a vertical cylinder with length equal to diameter, the required dimension is 68 m, which is greater than the crater bottom diameter. There is an added reason why such a geometry is unlikely. Heating of groundwater near the magma body is only possible if heat can penetrate the required distance in the time available; the repose period before the 18.10 h explosion was 50 min, and thermal waves can only travel distances of the order of a few tens of centimetres in rock in this time[16].

A long, cylindrical conduit feeding the vent is the geometry which maximises the chances of groundwater being involved in the explosions. Ngauruhoe stands ~950 m above the surrounding topography; if a vertical length of, say, 500 m were involved, the excavated cylinder needed to provide both the juvenile and nonjuvenile debris for all five major explosions would have had a diameter of 32 m. Figure 3 shows the situation envisaged: a long, cylindrical conduit about 20 m in diameter containing vesicular magma of mean density ~1,500 kg m^{-3}, cooled at the surface and chilled at its contact with the cone material, contains

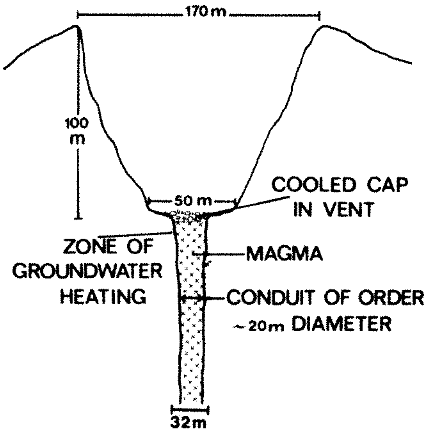

Fig. 3 Schematic of proposed cross-section through crater region of Ngauruhoe before the 18.10 h explosion.

clasts which have fallen into the conduit from earlier activity. Water in these clasts and in the cone material immediately surrounding the magma is heated to near-magmatic temperatures and, together with exsolved juvenile magmatic water, drives the explosion when the cap over the vent fails. It is not possible to estimate accurately the relative contributions from magmatic water and groundwater. The 18.10 h explosion must have removed a rock layer some 5 m thick from the conduit walls to satisfy the observed mass. Only a small fraction of this rock and its pore water could have been heated by conduction in the 50 min before the explosion, although water and steam in interconnected fissures may have been heated by convection. However, in the one to two seconds after the failure of the magma cap, that is during the period of acceleration of large blocks, rapid turbulent mixing of small fragments of disrupted magma and released groundwater would have taken place. It was shown above that up to 50% of the mass of disrupted magma could interact thermally with the evaporating water on this time scale. If all the available water in the wall rock layer were involved in the explosion, a water content of 2–3% in this rock would be consistent with the expected water contribution from the magma, as noted above.

In the above treatment we have not found it necessary to invoke the rapid mixing of juvenile material with groundwater that might lead to flash boiling[23]. This mechanism has been proposed as a means of creating pressures of the order of 3–5 kbar, similar to the pressures deduced for high velocity explosions[17] by applying the Bernouilli equation. Our analysis does not require such high pressures to initiate the explosion process.

Eruption column behaviour

The rise height, h, of the convective plume from the 18.10 h explosion can be computed from a formula for convective rise from an instantaneous source and compared with the observed height: h, in metres, is given[24] by

$$h = 1.87 Q^{1/4} \tag{9}$$

where Q is the released heat energy in joules. The 18.10 h event emitted[2] 2×10^8 kg of rock, of which about half was juvenile (that is, hot and able to contribute to driving convection). However, about half of the material injected into the plume was lost almost immediately (after 12–15 s) by the partial collapse of the column to form pyroclastic avalanches[2]. Thus, the heat available to drive convection is estimated to have been a maximum of 4×10^{13} J, leading to a predicted column height, h, of 4,700 m. The observed value[2] was at least 4,000 m above the vent, the discrepancy implying an efficiency of heat utilisation of about 50%.

This may be compared to values much closer to 100% found for maintained plumes produced by other eruptions[25] and also to the value found for a maintained plume at Ngauruhoe. During part of the gas-streaming eruptive phase, lasting from 13.25 to 13.45 h, prior to the series of discrete explosions, a 10 km high (above crater) convecting column existed[2] over Ngauruhoe. The equivalent of equation (9) for continuous column convection is[24,25]

$$h = 8.2 \dot{Q}^{1/4} \tag{10}$$

where h is the column height in metres as before and $\dot{Q}$ is the rate of release of heat energy in Watts. A 10 km column height implies a mass eruption rate of 3.2×10^6 kg s^{-1} (ref. 25). The estimated volume[2] erupted in this cloud was 1.6×10^6 m^3 dense rock which, averaged over 20 min and using the density of andesite equal to 2,550 kg m^{-3}, corresponds to 3.4×10^6 kg s^{-1}, in good agreement with the above value, suggesting that the efficiency of conversion of heat to work was close to 80% in this case.

Vulcanian explosions are very common at the many active andesitic strato-volcanoes clustered along subducting plate margins. Some of these eruptions are more violent than those described from Ngauruhoe[8,26]. However, previous estimates of pre-explosion over-pressures have generally been too large, and this article demonstrates that relatively small pressures are probably adequate to produce the observed behaviour during this type of eruption. Basaltic andesite magmas have water contents comparable to those deduced for the explosions[21,22,28], and the involvement of groundwater is possibly a significant feature of the events, but not an essential feature as proposed by Schminke[27]. It is not necessary to invoke violent mixing of juvenile material and groundwater on a large scale to explain the explosions though such mixing on a small scale may be a significant feature of some vulcanian explosions. Eruption cloud heights can be related to the rate of release of juvenile material during steady gas-streaming activity, and to the amount of released material in discrete explosions, using the formulae found to apply to larger-scale eruptions[25].

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Concentrations of metals in very small volumes of soil solution

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A new method of sampling very small amounts of soil solution (0.3 g) shows that soil solutions contain high concentrations and unusual proportions of metals. In the soils studied, the solution is close in both metal proportions and total metal mass to what may be taken up annually by the growth of plants at the sites sampled. Composition of soil solution varies seasonally and with depth in soil.

A FUNDAMENTAL problem in soil science and plant nutrition has been the determination of the true chemical composition of the films of solution which surround particles of normal, unsaturated soils and which are in contact with the roots of growing plants. Attempts to determine metal concentrations in soil solution have been hampered because conventional analysis requires large amounts of solution, and the collection of large samples generally alters the composition of the solution[1].

A method for sampling very small amounts of solution from undisturbed, unsaturated soils in the field was used for this study, and techniques originally used in nuclear geochemistry have been used to analyse the samples. This has the advantage that much smaller samples (roughly single-drop size) may be analysed to greater accuracy for both major metals and related trace metals than has previously been possible.

Field selection and sampling method

The soil solution is rapidly blotted onto a stack of specially cleaned absorbent paper. In the field, the sampler cleared a flat space at an appropriate horizon in the soil, and placed a porous-plastic (Nuclepore) filter on the soil surface. Then, wearing an acid-cleaned polyethylene glove, the sampler pressed with his thumb a stack of six specially cleaned and weighed 4-cm filter paper disks tightly against the plastic filter for a few seconds. The stack was put into a clean Teflon bottle and tightly sealed for reweighing in the laboratory. Rare contamination of the solution samples with particles from the soil was revealed by easily visible brown staining of the white absorbent paper, and proved in all cases to be associated with rupture of the plastic filter. The few such samples were discarded.

Two published reports[2,3] have proposed this sampling method in principle, and results have been reported by Schuffelen *et al.*[4] of a study using a similar sample collection method.

The study site, Thompson Canyon, comprised an area of 12.2 km^2 in the northern part of Yosemite National Park in the central crest region of the Sierra Nevada of California at an altitude of ~2,900 m (meadow surface). The area was selected because of freedom from disturbance by human activity, uniformity of rock (quartz monzonite) which has provided material to the soil since glaciation 10,000 yr ago, the fact that all water entering the watershed comes from precipitation, and the rather simple and stable assemblage of plant and animal species. The watershed has been used since 1970 for studies of geological and biological fractionation of metal families in terrestrial food chains[5-9].

Methods of analysis and soil sampling

Metals determined in the samples were the alkali metals K, Rb and Cs and the alkaline earth metals Ca, Sr and Ba. These metals were selected because the members of each family exhibit a narrow but definite range of behaviour in nature owing to similar electronic properties and varying ionic size. The metals have widely varying geochemical abundances, and most have been used extensively in soil science investigations. Na and Mg were not suited to the same analytical techniques as the six metals reported in this study.

Stable isotope dilution mass spectrometric analysis was used for all determinations. It is the most sensitive and accurate method known for determining very small amounts of many metals; the extremely small samples (~0.3 g solution) collected in this study were readily analysed for the six metals using this technique. The most extensive development and application of the technique has been in the field of nuclear geochemistry, and its use in this study is possible largely through its refinement for analysis of lunar material[10]. A known amount of isotopically enriched tracer was equilibrated with the sample so that the resultant isotopic ratio was a mass-weighted average of the isotopic composition of the enriched tracer and the natural isotopic composition of the sample. This resultant ratio was measured on a solid-source, thermal-emission mass spectrometer, and the sample mass calculated.

Seventeen samples of soil solution were analysed. These were taken from three localities (total of five horizons). Soils selected (entisols) were (1) a humus-rich soil in the centre of the broad, deep meadow in the bottom of the glacial valley, sampled in a single near-surface zone containing sedge roots; (2) a soil (humus-rich near the surface but less deeply developed than the meadow soil) perched on a narrow hillslope terrace, from which solution was sampled at both 5-cm (growth zone of shallow-rooted herb and sedge plants) and 30-cm depths (zone of deeper rooted plants such as large lupine); (3) a soil on the boundary of

Table 1 Metals in mid-meadow soil-solutions compared to phreatic water, stream water, and average snow (parts per 10^9)

Metal	4 mid-meadow soil-solutions	Phreatic water, same locality	Stream water, same locality	Snow*
K	7,000	11.0	62.0	9.7
Rb	71	0.17	0.26	0.042
Cs	11	0.005	0.011	0.0048
Ca	1,740	530	380	18
Sr	32	13	7.4	0.15
Ba	64	6.5	1.2	0.22
K/Rb	98	66	240	230
K/Cs	630	2,300	5,600	2,020
Ca/Sr	54	41	52	109
Ca/Ba	27	82	310	82
K/Ca	4	0.022	0.16	1.8

*Vertically integrated snowpack at maximum depth, 90% of total annual precipitation.

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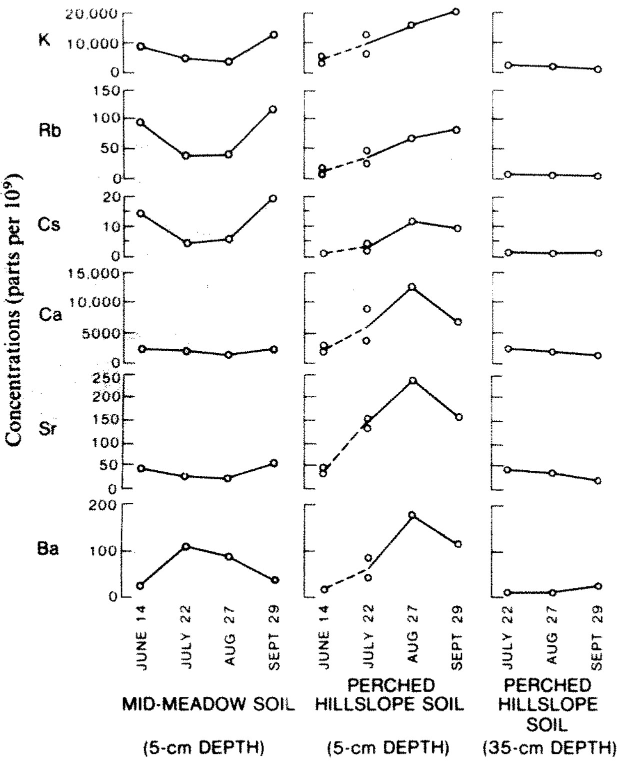

Fig. 1 Variation through the growing season in the metals composition of soil solutions: well developed soils in mid-meadow and on a hillslope platform (concentrations in parts per 10^9).

conifer forest and meadow, supporting both conifers and meadow plants.

For sample preparation the filter disks were leached of sample using 20 ml of 0.1 M, highest purity HCl for several hours at 55 °C in an acid-cleaned quartz centrifuge tube and then centrifuged to precipitate fibres. Aliquots of this solution were removed with a pipette, isotopically spiked, and analysed for metals.

Tracers for all six metals were mixed together and added to aliquots of solutions made up from soil solution samples. The six metals were analysed in the mass spectrometer without chemical separation. Actual metal masses that were determined ranged from 160×10^{-9} g for K to 0.02×10^{-9} g for Cs, with blanks always less than 10% of these masses. To minimise contamination, special precautions were taken; these involved clean rooms, ultra-pure reagents, ultra-clean apparatus, and low contamination procedures in the field[11].

The accuracy and precision of the soil solution sampling and analytical method were tested using a homogeneous stock solution containing metals in concentrations very similar to those of the soil solution. The stock solution was sampled in two different ways. In one, an aliquot was taken directly from the bottle, spiked, and analysed with minimum treatment. In the second, a sample was blotted onto cleaned filter paper through a Nuclepore plastic filter and removed by leaching, a method analogous to the method of sampling the soil solution in the field. The data (not presented here) show that passage of moisture through the plastic filter and subsequent acid extraction from the absorbent paper do not introduce any large errors: results obtained using the two ways of sampling the known stock solution agreed within a few per cent.

In addition, reproducibility of results from actual field samples was tested by two methods. (1) Two samples of soil solution were taken in rapid succession directly from the cleared soil surface in the field, only a few centimetres apart (results plotted as double data points in Fig. 1, 14 June collection, perched hillslope soil; Cs and Ba analyses failed on one of the samples). At the same site, a soil sample (1,500 g) was thoroughly stirred in a clean polyethylene pan and two solution samples were collected from it in rapid succession (plotted as double points in Fig. 1, 22 July collection). As shown in Fig. 1 the variations in the duplicates are important fractions of the total masses, especially for Ca, but generally both values in each duplicate pair are close, even for Ca, and both fit well within the seasonal trend plotted. The variation must be attributed to inhomogeneities in the soil on a scale of a very few centimetres.

In a primitive version of the method used in this study[12] the bottom disk of the stack of absorbent papers was in direct contact with the soil and was discarded. This method introduced errors because the heavier alkaline earth metal ions (Sr and Ba) were preferentially retained in the bottom disks of the stack, through which the sample first passed. This finding led to the use of the porous plastic filter between soil and absorber during collection.

To clean the absorbent paper the Whatman No. 40 ashless, cellulose-acetate filter paper disks (5.5 cm) were batch-cleaned by leaching for 24 h in each of three successive 55 °C baths of 25% HCl solutions of increasing purity (analytical reagent grade, G. Frederick Smith distilled, National Bureau of Standards distilled). Moderately thorough water rinses (to *p*H 4 or 5) were used between each acid bath; highest purity rinse water was sucked through a few disks at a time on a Buchner funnel. The disks were vacuum dried between each acid bath. The series of HCl leachings was followed by a similar series in 0.5% highest purity HF and in 1% NH_4Cl at *p*H 7. The final rinse, after the NH_4Cl, was very sparing, so that some NH_4^+ would be left on the paper (E. E. Dickey, personal communication). The 0.4 μm pore size Nuclepore filters were cleaned in the same way as the papers. The HF and NH_4Cl steps reduce by 10-fold a troublesome Ba analytical blank, compared to that for a simple HCl sequence[12].

Results and discussion

Compositions of soil solution are quite different from those of any phreatic and stream waters in the watershed and elsewhere[13–15]. Metal concentrations in soil solution samples taken near the soil surface were as much as 1,000 times higher than those in phreatic water or stream samples collected at the same location (Table 1). Soil solution is the only type of water in the

Table 2 Moisture content of soils from which solution was sampled

Date	Mid-meadow soil (5 cm depth, 19% organic)	Perched hillslope soil (5 cm depth, 7.1% organic)	Perched hillslope soil (30 cm depth, 2.7% organic)	Forest-meadow boundary soil (5 cm depth, 6.3% organic)	Forest-meadow boundary soil (30 cm depth, 3.3% organic)
14 June	1.14	1.05	–	0.96	0.61
22 July	ND	0.92	0.60	–	–
27 Aug.	0.98	ND	ND	–	–
29 Sept.	1.05	0.98	0.63	1.00	0.58

Values represent mass of moisture as fraction of mass of air-dry soil. ND, not determined; –, no sample collected.

Table 3 Comparison of metal availability in a meadow soil* by two different measures

Metal	Exchangeable bases† (mEq per 100 g)	In soil solution‡ (mEq per 100 g)
Na	1.3	–
K	0.11	0.0033
Ca	0.95	0.0016
Mg	0.23	–
Total exchange cations	21	–
K/Ca	0.12	2.1

*This soil (49% water) and soil solution collected in 1973.

† Exchangeable bases measured with NH_4^+ at *p*H 7 by US Soil Conservation Service, Riverside, California.

‡Blotting method of this study.

watershed that has higher concentrations of alkali metals than alkaline earth metals. Stream water, phreatic water, and snow all contain more alkaline earths than alkalies. In no other natural waters measured are the concentrations of Rb and Cs, either absolutely or relative to K or to the alkaline earth metals, as large as they are in soil solution.

Solutions were collected at various times in the summer, at various soil depths, and from various soil types. The data are plotted in Fig. 1. In solutions near the surface (5 cm depth) of humus-rich soils, the concentrations of alkali metals (K, Rb, Cs) are highest in the autumn (mid-meadow and perched hillslope soils). In samples taken at greater depth below surface (30 cm, more mineralic zones), the alkali concentrations are lowest in the autumn (perched soil). For these deeper zones the seasonal trend appears less distinct in Fig. 1 only because the absolute concentrations and their changes are smaller, and because the scale of the plot has not been expanded.

Three possible factors influencing the seasonal soil-solution composition are the following: (1) metal uptake and release of metals by the standing crop of plants; (2) through-flow of solutions having different metal contents, and (3) uptake and release of metals by humus and clay in soils, in response to temperature, *p*H, or other physical and chemical factors. As for the effects due to the growth of plants, the mass of metals in soil solutions above the water table in mid-meadow is comparable to the mass of metals in the leaves of the standing crop of sedge (Table 4). Metal ratios in sedge leaves vary strongly with time of season[16], but these variations may represent only internal redistribution of metals between the leaves and the root systems of these plants. Variation in soil-solution composition caused by through-flow of soil solutions of variable composition is unlikely in these poorly drained soils, except in the earliest snow-melt part of the season. *p*H and temperature may affect the release of metals from humus and clay; *p*H was between 4 and 5 at all times of the season for all soils and average soil temperatures can rise measurably in mid-season. Equilibration times needed to replace metals in soil solution from the humus and clay reservoirs have not been determined for the soils of this study.

Table 4 Comparison of masses of metals in soil solution and in annual plant productivity

Metal	In soil solution	In annual plant productivity
K	7,000	14,000
Rb	71	81
Cs	11	8.5
Ca	11,750	5,200
Sr	32	96
Ba	64	81

Mid-meadow soil; mass of metals in solution to 10 cm depth; plant values from standing crops of sedge leaves; from unpublished data of Patterson and Elias; data in g per hectare.

Apart from seasonal variations, the major differences between the soil solutions collected near the soil surface (humus-rich zones) and those collected at greater depths (mineralic zones) are the following: (1) the metal concentrations are generally higher in the near-surface samples, and (2) alkali metals are more abundant relative to the alkaline earth metals in the near-surface samples. K/Ca ratios are most commonly greater than unity in the deeper zones. It is empirically true in samples from the field area of this study that, in direct correspondence to the composition of the shallow and deep soil solution, the leaves of shallow-rooted plants (sedges) have higher K/Ca ratios than those of more deeply rooted plants (conifers)[16]. Roots of these plants were present in the respective zones at the actual sampling sites.

The moisture contents of the soils sampled in this study (Table 2) are high and do not vary greatly. There are distinct differences between shallow, organic-rich soils and deeper, mineralic soils, but these subalpine soils do not dry out through the summer as do soils in other environments. The effect of lowered moisture content on the absolute or relative concentrations of the two families of metals determined are not known from the experiments of this study; it is accepted that changes in moisture content alter the distribution of monovalent and divalent metals between soil and solution[17], but it seems that the seasonal variation in solution metal composition observed in this study (Fig. 1) is to be associated with other factors. It is not known whether samples large enough for analysis could be collected by the blotting technique from soils much less moist than those of this field area.

The data presented in Table 3 show that in the particular non-agricultural soils of the study area the reservoir of metals contained in soil solution is much smaller than the reservoir available as exchangeable cations. In the study area the mass of metals in the soil solution is approximately equivalent to the mass turned over by plants in the annual growth cycle (Table 4). The proportions of the metals in the solution reservoir are very different from those in the exchangeable cation reservoir: in soil solution, the alkali metals seem to be relatively more abundant than the alkaline earth metals, whereas in the exchangeable cation reservoir the reverse is true (Table 3, K/Ca values 2.1 and 0.12, respectively).

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Crystal structures of *Streptomyces* subtilisin inhibitor and its complex with subtilisin BPN′

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Crystal structure of a microbial protein proteinase inhibitor SSI (Streptomyces *subtilisin inhibitor) and its complex with subtilisin BPN′ were solved at 2.6 Å and 4.3 Å resolution respectively. The mode of binding to the proteinase of SSI is compared with that of bovine pancreatic trypsin inhibitor, soybean trypsin inhibitor and the substrates. Stereochemical considerations for the possible evolutionary relationship between SSI and pancreatic secretory trypsin inhibitor are presented.*

MANY protein proteinase inhibitors from animal and plant tissues have been extensively studied[1]. However, bovine basic pancreatic trypsin inhibitor[2–4] (Kunitz, BPTI) and soybean trypsin inhibitor[5,6] (Kunitz, STI) are the only protein proteinase inhibitors which have been studied by X-ray diffraction. *Streptomyces* subtilisin inhibitor (SSI), isolated from *Streptomyces albogriseolus* by Murao and Sato[7,8], is one of the few well characterised microbial protein proteinase inhibitors. SSI has been characterised chemically and spectroscopically and by the X-ray diffraction studies carried out in a collaborative study involving nine laboratories, led by K. Hiromi (summarised in ref. 9). Unlike classical trypsin inhibitors[1], SSI strongly ($K_{\rm I} \simeq 10^{-10}$ M)[10] and specifically[8] inhibits bacterial alkaline proteinases (such as subtilisin BPN′), although it also weakly inhibits α-chymotrypsin and trypsin ($K_{\rm I} \simeq 10^{-6}$ and 10^{-4} M, respectively)[11,12]. SSI is a stable dimer[13] (I_2) composed of two identical subunits each of molecular weight 11,483 (ref. 14). In the trigonal crystals ($P3_121$, $a = b = 40.6$ Å, $c = 115.2$ Å) of SSI analysed here, the two subunits are related by a crystallographic diad[15].

The inhibition of subtilisin BPN′ by SSI occurs when one subunit (I) of SSI inhibits one molecule (E) of subtilisin[16,17], and the molecular species of the SSI · subtilisin complex in solution was characterised as E_2I_2 (ref. 18). The orthorhombic crystal of this complex ($I222$, $a = 77.5$, $b = 186.9$, $c = 69.6$ Å), analysed here, contains half the complex molecule (EI, MW ~ 39,300) in an asymmetrical unit[15]. Furthermore, the amino acid sequence of the subunit of SSI, a single polypeptide chain with two disulphide bridges, was determined by Ikenaka *et al.*[14]. Chemical evidence, including the unusual susceptibility of the Met-73–Val-74 bond to trypsin and α-chymotrypsin, led these workers to propose that this was the susceptible bond[14]. We have shown here that this hypothesis is correct by crystal structure analysis of the complex molecule.

Structure of SSI

The structure analysis of SSI at 4 Å resolution was reported previously[19]. For 2.3-Å resolution study, about 13,000 reflections (including Friedel pairs) were collected for each of the three heavy-atom derivatives, $(NH_4)_2Hg(SCN)_4$, $(NH_4)_2Pt(NO_2)_4$ and ethylmercuric thiosalicylate, for which the root-mean-square (r.m.s.) heavy-atom structure factor/r.m.s. lack-of-closure error was 38.6/19.3; 34.9/18.6; and 36.8/19.6 electrons, respectively. Detailed analysis of the statistics, however, revealed that the phasing between the 2.3-Å and 2.6-Å spheres was unreliable so that the maximum resolution of the map was 2.6 Å. The map was interpreted using a half-mirror device[20] and the 1 Å = 2 cm Kendrew model. For rapid measurement of the coordinates of the wire model, a semi-automatic device using two He–Ne laser tubes and magnetic encoders was developed[21]. Details of the structure analysis will be published elsewhere[22].

A schematic drawing of the subunit has been previously presented[23]; the electron densities are easily interpretable except for the N-terminal four residues almost missing) and the region Ala-62–Met-70, where only the approximate course of the main chain can be traced. The size of the subunit is about 25 × 35 × 40 Å, whereas that of the dimer shown in Fig. 1 is about 30 × 40 × 65 Å. The subunit has a five-fold antiparallel β-sheet twisted as usual[24], and two short α-helices (α_1(46–57) and α_2 (100–107)). The topology of the β-sheet in relation to the primary sequence is such that the five β-strands are arranged from the top to the bottom of the molecule thus: β_5 (residue numbers 89–97)↑, β_4 (77–87)↓, β_1 (10–19)↑, β_2 (29–35)↓, and β_3 (40–42)↑, where arrows indicate the polarity relationship between the polypeptide chains. The same β-sheet topology is seen elsewhere only as part of the variable region of immunoglobulin[25,26], in which the 30–40 β-strand corresponds to the β_1 strand of SSI.

According to Levitt and Chothia[27], SSI subunit belongs to the "class II" or "all β" type of proteins. Most of the non-crystallographic predictions[9] on the states of some specific amino acid residues have proved qualitatively correct. These include the nature of the peptide NH (refs 28,29), conformation of S–S bridges (M. Nakanishi, unpublished observation), and the states of tryptophyl[30,31], tyrosyl[30,32], histidyl[32,33], and methionyl residues[32]. Detailed evaluation of these results will be published elsewhere[22].

The subunit–subunit interface

The interface is formed by the stacking of a β-sheet of one subunit on top of a β-sheet of the other subunit, related by a diad. The planes of the two β-sheets are roughly parallel (Fig. 1*a*), whereas the β-strands in the opposite sheets are inclined at about 30° to each other (Fig. 1*b*). Similar stacking of β-sheets at the subunit–subunit interface has been previously reported only at the dimer–dimer interface of concanavalin[34] and in the monomer–monomer interface of glyceraldehyde-3-phosphate dehydrogenase related by the 'P' axis[35]. In the former case, the β-strands in the opposite sheets are inclined to each other in about the same way as in SSI, but in the latter case, they seem to

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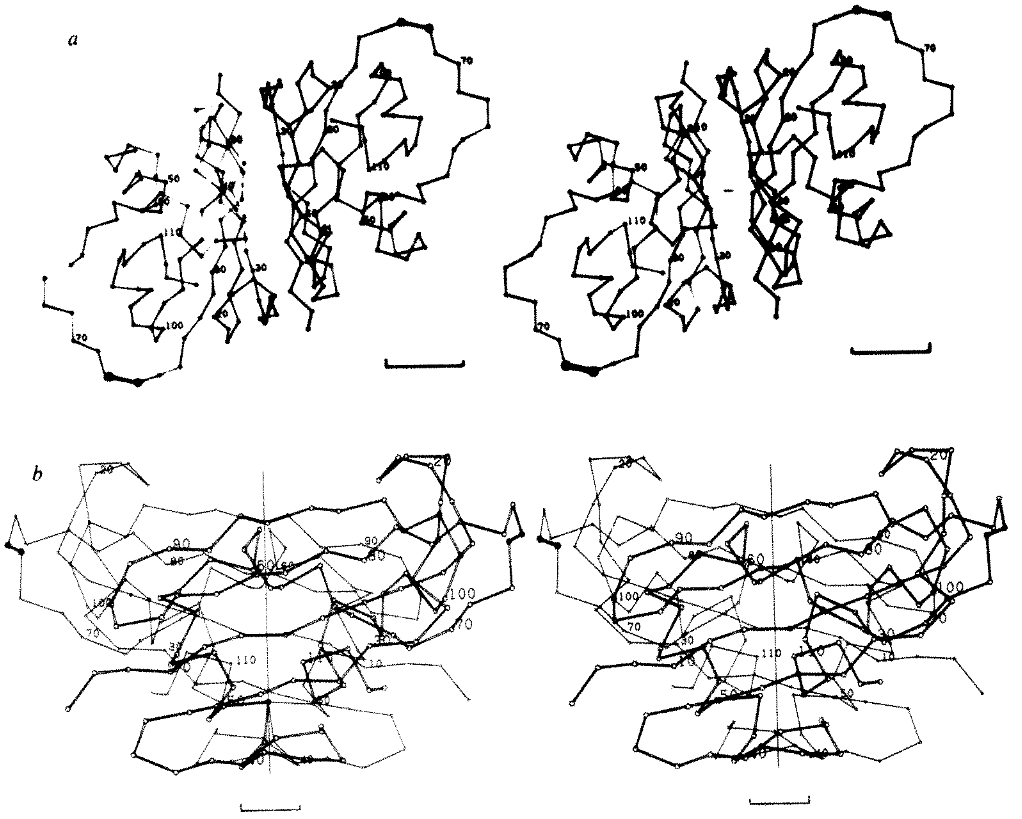

Fig. 1 α-Carbon chains of a SSI dimer viewed *a*, along the diad and, *b*, perpendicular to the diad. The reactive site Pl, Pl′ residues are marked. Scale bars represent 10 Å and 5 Å, respectively, for *a* and *b*.

run almost parallel. All the 48 intersubunit contacts of less than 3.8 Å, except for three possible hydrogen bonds, are non-polar in agreement with the observed susceptibility of the dimer to SDS (ref. 36). An interesting feature of the side chain packing in the interface is the straight side chain of Arg-90 held tightly in a pocket formed by Thr-15, Ala-22, Pro-27 and Leu-79 of the opposite subunit, and constituting an effective device for correct alignment of the subunits.

Of the 15 residues participating in the subunit–subunit interaction, only one replacement residue, Thr-34 to Asn, is seen in the closely related dimeric inhibitor, plasminostreptin (isolated from *Streptomyces antifibrinolyticus*)[37–39], which has 64% overall homology with SSI. This suggests that the purpose of the dimeric structure is the preservation of its own function and or stability. Inspection of the model shows that all the amino acid side chains replaced in plasminostreptin can be accomodated in the structure of SSI. SSI and plasminostreptin are, therefore, homologous proteins.

The reactive site and the C-terminal region

The electron density around the reactive site residues (Cys-71 to Tyr-75) is very high indicating the rigidity of this region as in BPTI[4] and STI[6]. The region seems to be tightly held by the β_4-strand embedded in the β-sheet and by the 71–101 disulphide bridge which, in turn, is fixed to the core of the subunit through helix α_2 and the C-terminal residues. The C-terminal four residues Val-110, Phe-111, Ala-112 and Phe-113 (the 111 and 112 order has been reversed (T. Ikenaka, personal communication)), can be selectively removed from intact SSI by treatment with carboxypeptidase A (M. Sakai, M. Odani & T. Ikenaka, in preparation). This derivative, SSI-A, still weakly inhibits subtilisin BPN′. However, the inhibition is only temporary if an excess of the enzyme is present. The normal inhibitory activity was almost completely recovered by adding a 30-fold excess of the C-terminal deca-, nona- or octapeptides to SSI-A. These observations, together with the UV difference

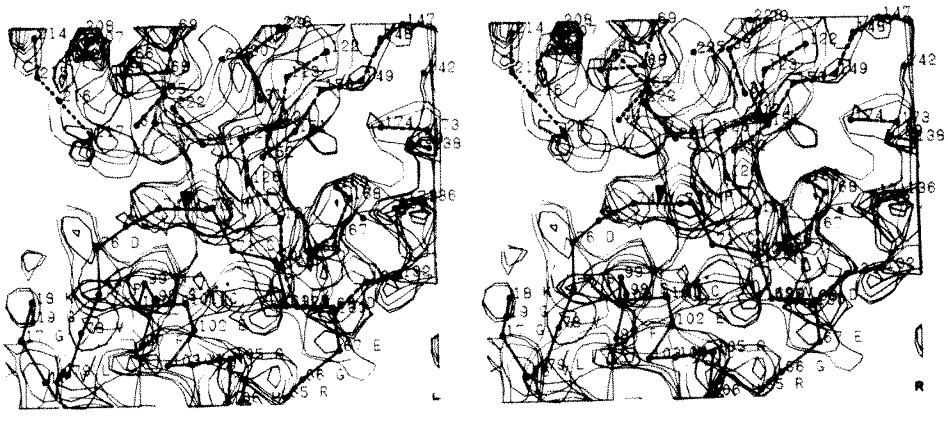

Fig. 2 α-Carbon chains of SSI (solid lines) and subtilisin BPN′ (broken lines) in the region of contact fitted into a 4.3-Å resolution electron density map of the SSI · subtilisin complex. The scissible bond is marked by ▼.

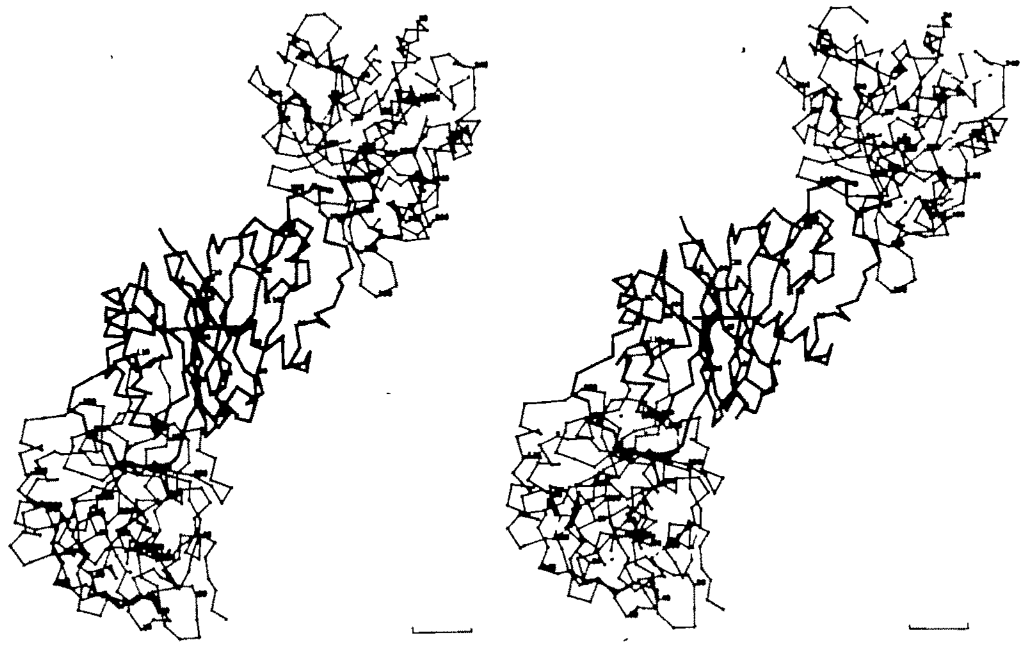

Fig. 3 α-Carbon chains of a complex of SSI dimer (solid bonds) with two subtilisin BPN′ (open bonds) The diad (horizontal line in the centre) is inclined to the plane of the paper at an angle of 60°. Scale bar represents 10 Å.

spectra, suggested that SSI-A has a somewhat loosened structure which is susceptible to proteolytic attack, but that the structure of intact SSI is almost fully restored on addition of the C-terminal oligopeptides. The C-terminal four residues make 43 inter-atomic contacts of less than 4 Å with the rest of the molecule, 93% of them being non-polar; this may explain the destabilisation of the molecule when they are removed.

Comparison of main-chain conformations of the reactive site residues and the substrate

Table 1 compares the main-chain torsion angles in the reactive sites of BPTI, STI, SSI and a hypothetical substrate[40] when bound to subtilisin BPN′. For BPTI and STI the values were obtained from the published refined coordinates[1,6]. The values for SSI, although not yet refined, are probably correct within 15°. The values for the substrate are based on provisional coordinates. Between BPTI and SSI, the angles for P_2, P_1, P'_1 and P'_2 agree within experimental error, despite the completely different amino acid sequences at the reactive site and the different proteinase specificity (BPTI does not inhibit subtilisin BPN′). For P_2 and P_1 the agreement extends to STI as well. The difference in P'_2 parameters between STI and SSI clearly reflects different chain conformation. The P'_1 parameters of STI present a problem. The combination of ϕ and ψ is energetically unfavourable, and Sweet *et al.*[6] noticed that, in the given orientation of the peptide bond between Ile-64′ and Arg-65′, there was no suitable hydrogen bond for NH(65′). Re-examination of the map shows that $\phi(P'_1)$ may well be $\sim 180°$ (J. Janin, personal communication). For P_3, the trypsin inhibitors do not share a common conformation with SSI. This reflects the fact that, in the trypsin inhibitors, P_4, P_5, ------ are not on the surface of the enzyme, but those in SSI are still on the surface.

Comparing SSI and the hypothetical substrate, the overall agreement (except for $\psi(P'_2)$) is clear, if allowances are made for the appreciably large errors in the latter coordinates. The difference in $\psi(P_1)$ and $\phi(P'_1)$ might be significant, but detailed study must await a high resolution map of the SSI · subtilisin complex. At the resolution now obtainable, there is no clear indication of any change in SSI conformation on complex formation. The structure of the hypothetical substrate was deduced from X-ray difference Fourier studies on the binding of various peptide chloromethyl ketones[41] and polypeptides[40] to subtilisin BPN′. Apparently because of weak P_1 specificity (compared to trypsin), the results were controversial. Despite these difficulties, the present comparison strongly suggests that the productive binding mode of substrates to subtilisin proposed

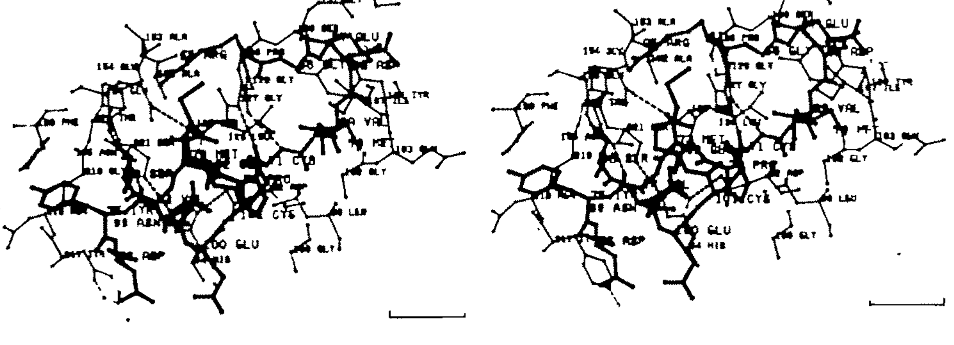

Fig. 4 The region of contact of a model of the SSI (in solid bonds and larger characters) and subtilisin BPN′ (in open bonds)[43] complex constructed as for Fig. 3. All residues relating to the intermolecular short contacts of less than 4 Å are shown The bonds for Arg-65′–Met-70′ are striped because the corresponding electron density in the SSI crystal is very weak. Scale bar represents 5 Å.

by Kraut *et al.*[40] is essentially correct and that, as in the case of trypsin inhibitors[3,6], the binding mode of a protein proteinase inhibitor (SSI) to subtilisin is, at least, closely related to that of substrates.

Comparison of overall conformation in BPTI, STI and SSI

The similar conformations of the reactive sites of the three inhibitors prompted us to investigate whether the similarity extends to any other part of the molecule. Despite the large difference in length (BPTI, STI and SSI have 58, 181 and (2×)113 residues, respectively) and apparent heterology in primary sequences, this seemed relevant, as three-dimensional structure is one of the properties which is probably most highly conserved during evolution. The coordinate systems of BPTI and STI were translated and rotated by a least-squares procedure to optimise the overlap between the corresponding main-chain atoms of the reactive site residues (P_2 to P'_2 for BPTI, P_2 to P'_1 for STI) and those of SSI. The general non-relatedness between SSI and BPTI (or STI) was clear, although there is some topological resemblance between residues 6–43 of BPTI and residues 60–103 of SSI (details will be published elsewhere)[22]. Most probably, the three inhibitors have evolved convergently from independent ancestral proteins, for the common purpose of inhibiting serine proteinases, and resulting in a similarity of the reactive site alone.

Table 1 Reactive site residues and main-chain conformations of BPTI[3], STI[6] and SSI

	P_3		P_2		P_1		P'_1		P'_2	
	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
BPTI	−86	−28	−64	152	−116	87	−135	166	−116	87
STI	−46	−21	−77	136	−89	85	(−135)	(−115)	−175	−179
SSI	−123	141	−52	134	−92	89	−117	169	−120	78
Hypothetical substrate	(−157)	(172)	(−84)	(162)	(−116)	(26)	(−62)	(136)	(−112)	(−70)
BPTI	Pro		Cys		Lys(15)		Ala(16)		Arg	
STI	Ser		Tyr		Arg(63)		Ile(64)		Arg	
SSI	Cys		Pro		Met(73)		Val(74)		Tyr	
Hypothetical substrate	Ala		Ala		Tyr		Val		Val	

Corresponding data (in parentheses) for a hypothetical substrate[40] productively bound to subtilisin BPN′ are only provisional. Dihedral angles within 35° of the corresponding ones in SSI are outlined.

It is interesting that there are already three distinct patterns of 'protein proteinase inhibitor fold', and the number of such folds is likely to increase, as there are many examples (such as soybean Bowman–Birk inhibitor) of compounds whose structure cannot be similar to any of the known folds, because they have a different pattern of disulphide bridging[42]. In contrast, the serine proteinases, the target molecules, show only two basic patterns of chain folding, those of trypsin and subtilisin.

Structure of the complex molecule

The mixture (~1:2) of SSI and purified subtilisin BPN′(EC3.4.21.14) (Nagase, lot. 7370935) in 0.05 M Tris-HCl buffer (*p*H 7.5) was separated from excess inhibitors and autodigested enzymes by gel filtration. The crystals were easily obtained by leaving the main fractions in a cold room[16]. The structure was solved at 4.3 Å resolution using two heavy-atom derivatives, $(NH_4)_2Pt(NO_2)_4$ and K_2PtCl_4, for which the ratio r.m.s. heavy-atom structure factor/r.m.s. lack-of-closure error for the 3,550 independent reflections was 16.4/7.6 and 15.7/7.3, respectively, and of which details will be published elsewhere.

The model shown in Fig. 3 was first constructed using a rough correspondence in reactive site conformation between SSI and a hypothetical substrate (Table 1), and then adjusted by fitting it to the electron density through computer graphics (Fig. 2). At this resolution, there is no sign of major conformational change on complex formation in either SSI or subtilisin BPN′. As in the case of trypsin inhibitors[3,6], the region of contact is small: of the 113 and 275 amino acid residues, respectively, composing SSI subunit and subtilisin BPN′, only 11 and 19 residues are in contact. Recent estimation of the thermodynamic parameters of the complex formation using the temperature dependence of K_I yielded a value $\Delta H = +4.2$ kcal mol^{-1} and $\Delta S = +57$ e.u. (at 25°C, *p*H 7.0)[10], showing that the process is entropy driven as is the case for other trypsin inhibitors[5].

The SSI–subtilisin interface

Figure 4 was obtained by the same procedure as for Fig. 3. Because the parameters defining relative translation and orientation of the two molecules were adjusted with reference to the electron density map, the nature of the model is semi-empirical. The model is based on the coordinates determined for the crystals of SSI alone and subtilisin BPN′ alone[43,44]. Thus, in the actual structure, induced fit could cause movement of, for example, Tyr-104 of the enzyme (as observed in the difference Fourier studies[40,41] on subtilisin BPN′). As in trypsin inhibitors[3,6], the sequence of P_4 (Met-70′, (prime indicates the inhibitor)), P_3 (Cys-71′), P_2 (Pro-72′) and P_1 (Met-73′) makes an antiparallel β-sheet with the Ser-125–Gly-127 sequence of the enzyme. A possible scheme for hydrogen bonding is shown. Discussion of the nature of the interaction between the susceptible bond and the active serine (Ser-221) must await high resolution studies.

There are two kinds of interaction which differ from those made by trypsin inhibitors[3,6]. First, P_4 (Met-70′), P_6 (Asp-68′) and P_7 (Glu-67′) are still on the surface of the enzyme. Although the precise mode of interaction involving these residues cannot yet be considered because of their flexibility in the SSI crystal, the proximity of P_4 to Gly-102, Glu-103 and Tyr-104 and of P_6 and P_7 to Ser-130 is clear. In the complex crystals, the electron density for the region Glu-67′ to Met-70′ is comparable with that for other regions (Fig. 2). We may tentatively conclude that the flexible loop (Glu-67′–Met-70′) of SSI becomes fixed on complex formation with subtilisin BPN′. We suggest that the P_4 and the subsequent (to the N terminus) residues may be important for the interaction of substrates with subtilisin BPN′. Robertus *et al.*[40] suggested that the S_4 site has a strong affinity for the phenylalanine side chain and that the site might have an important role in determining the cleavage point for the polypeptide substrate. P'_3 (Asp-76′) and the subsequent (to the C terminus) residues, on the other hand, are not in contact with the enzyme.

The second unique interaction made by SSI is located along the polypeptide chain close to the reactive site, through the 71′–101′ disulphide bridge. In this region, Ser-98′ is close to Asn-155 and Glu-156, whereas Glu-102′ is close to Glu-156 (Fig. 4). Let us call this site of interaction the 'secondary contact region', as opposed to the 'primary contact region' which is related to the reactive site residues (Glu-67′–Tyr-75′). The interaction through the secondary contact region is also present in the complex of BPTI with trypsin[3,4]. In this case, the residues Gly-36′, Gly-37′ and Cys-38′, which are close to the reactive site through the 14–38 disulphide bridge, are on the surface of the enzyme. A hydrogen bond is present between the peptide carboxyl group of Gly-37′ and the phenolic hydroxyl group of Tyr-94 (ref. 3). However, optimal superposition of BPTI on SSI (mentioned above) shows that the stereochemical relationship of the secondary site to the primary site is completely different

Table 2 Comparison of amino acid sequences of SSI[14] and plasminostreptin[37] with those of the PSTI family

[a]Homology(%)	0	0	23	2	20	59	100	82	62	95	56
				(P6)	(P5)	P4	P3	P2	P1	P1′	P2′
Residue no. (SSI)	65	66	67	68	69	70	71	72	73	74	75
SSI	R	G	E	D	V	M	C	P	M	V	Y
Plasminostreptin	V	R	G	D	V	A	C	T	K	Q	F
Acrosine inhibitor (porcine)	—	—	H	L	F	F	C	T	R	Q	M
Bdellin 3(leech)	—	—	—	—	—	V	C	T	K	E	L
PSTI (bovine)	—	—	E	V	N	G	C	P	R	I	Y
(ovine)	—	—	E	V	N	G	C	P	R	I	Y
(porcine)	—	—	E	V	S	G	C	P	K	I	Y
(human)	—	—	E	L	N	G	C	T	K	I	Y
Submandibular inhibitor I (canine)	—	—	—	—	—	A	C	P	R	L	H
II (canine)	—	—	—	—	—	M	C	T	M	D	Y
Avian ovomucoid inhibitor	— (17) G (14) (1)	— (17) K (14) (1)	— (17) D (8) V (3) E (2) (1)	— (18) V (9) M (2) E (1) T (1)	— (17) L (8) V (3) I (2) (1)	A (17) V (7) L (5) D (2)	C (31)	T (20) P (4) N (3) M (1) L (1) S (1) A (1)	K (7) M (7) L (6) E (5) R (2) D (1) A (1) S (1) Q (1)	E (21) D (8) A (2)	Y (16) L (10) F (2) H (1) D (1) Q (1)
Residue no. (PSTI)	—	—	12	13	14	15	16	17	18	19	20

[a]Homology(%)	28	90	100	0	0	97	0	100	64	0	0
	(P3′)										
Residue no. (SSI)	76	77	78	79	80	81	82	83	84	85	86
SSI	D	P	V	L	L	T	V	D	G	V	W
Plasminostreptin	D	P	V	V	V	T	V	D	G	V	W
Acrosine inhibitor (porcine)	D	P	I	C	G	T	—	N	G	—	—
Bdellin 3(leech)	H	R	V	C	G	S	—	D	G	—	—
PSTI (bovine)	N	P	V	C	G	T	—	D	G	—	—
(ovine)	N	P	V	C	G	T	—	D	G	—	—
(porcine)	N	P	V	C	G	T	—	D	G	—	—
(human)	N	P	V	C	G	T	—	D	G	—	—
Submandibular inhibitor I (canine)	E	P	I	C	G	T	—	D	H	—	—
II (canine)	R	P	L	C	G	S	—	D	G	—	—
Avian ovomucoid inhibitor	R (18) M (3) S (3) N (3) E (1) Q (1) H (1) K (1)	P (28) L (2) F (1)	L (16) I (9) V (6)	C (31)	G (31)	S (18) T (12) (1)	— (31)	D (31)	N (15) G (13) S (2) (1)	— (31)	— (31)
Residue no. (PSTI)	21	22	23	24	25	26	—	27	28	—	—

[b]Homology(%)	38	54	95	95	69	82	49	100	82	0	0
Residue no. (SSI)	94	95	96	97	98	99	100	101	102	103	104
SSI	E	R	V	F	S	N	E	C	E	M	N
Plasminostreptin	E	R	T	F	A	N	E	C	V	K	N
Acrosine inhibitor (porcine)	—	K	S	Y	A	N	P	C	I	F	C
Bdellin 3(leech)	—	V	T	Y	D	N	E	C	L	A	C
PSTI (bovine)	—	V	T	Y	S	N	E	C	L	A	C
(ovine)	—	V	T	Y	S	N	E	C	L	L	C
(porcine)	—	I	T	Y	S	N	E	C	V	L	C
(human)	—	D	T	Y	P	N	E	C	Y	L	C
Submandibular inhibitor I (canine)	—	K	T	Y	S	N	E	C	M	L	C
II (canine)	—	K	N	Y	S	N	K	C	S	F	C
Avian ovomucoid inhibitor	— (31)	K (17) V (13) (1)	T (30) (1)	Y (29) H (1) (1)	G (11) S (5) D (5) A (3) N (3) T (2) M (1) V (1)	N (24) S (3) — (3) H (1)	K (17) E (13) (1)	C (31)	N (16) L (9) M (3) V (1) S (1) (1)	F (17) L (12) (2)	C (31)
Residue no. (PSTI)	—	29	30	31	32	33	34	35	36	37	38

The comparison is made for *a*, the primary contact region and *b*, the secondary contact region (see text). The Table is an extension of that made by Laskowski, Jr[48]. For the avian ovomucoids[48] frequencies of appearance are shown in parentheses. Somewhat arbitrary homology relationships T = S, P = F; R = K, V = L = I were assumed. The cystines corresponding to Cys-71 and Cys-101 of SSI are connected in all the inhibitors. Solid outlines are for homologies involving SSI and dotted outlines are for those involving plasminostreptin only.

for the two inhibitors[22]. In the case of STI, which has no disulphide bridge close to the reactive site, there cannot be a similar secondary contact region, although some interactions other than those at the primary site are present[6].

Relation to pancreatic secretory inhibitor

There is evidence for the close evolutionary relationship of pancreatic secretory (Kazal) trypsin inhibitors (PSTI)[45], acrosine inhibitors[46], canine submandibular inhibitors[47] and avian ovomucoids[48]. Let us provisionally call this 'family' of trypsin inhibitors the PSTI family. Moreoever, Ikenaka *et al.*[14] discovered a homology between SSI and PSTI. To a recent exhaustive list of the PSTI family made by Laskowski[48], data for SSI[14] and plasminostreptin[37] have been added (Table 2). In the reactive site (the primary contact region) the homology between the SSI sub-family (including plasminostreptin) and the PSTI family extends over 19 residues, ranging from Glu-67′ to Val-85′ of SSI. Another homologous region exists between Val-96′ and Glu-102′ of SSI[14]. In both the PSTI family and the SSI sub-family, the cystine corresponding to Cys-71′ of SSI is connected to that corresponding to Cys-101′ of SSI. Surprisingly, this second homologous region is exactly the same as the secondary contact region mentioned above. In SSI, the side chain of Glu-100′ is exposed to the solvent, making no contact with either subtilisin or other parts of SSI. Thus the frequent replacement of this residue by a lysine having opposite charge is understandable. Asn-99′, having no contact with subtilisin, seems to act as a spacer between the primary and secondary contact regions. The persistent conservation of this asparagine residue might reflect such a role. The replacement of this residue by histidine inactivates the inhibitor (Laskowski, personal communication).

Ser-98′ makes contacts with Asn-155 and Glu-156. The replacement of this residue seems to be mostly restricted to the residues with stereochemically similar side chains. The same restriction seems to apply to Glu-102′ the other residue making direct contact with subtilisin. The other highly conserved residues, Phe-97′ and Val-96′, make no contacts with subtilisin. However, Phe-97′ seems to have the important role of fixing the secondary contact region to the rest of the molecule, with its benzene ring being tightly held by Met-103′, His-106′ and Arg-95′. Based on these findings it is very tempting to conclude that the inhibitors of the SSI sub-family and those of PSTI family are evolutionarily related despite their large phylogenetic distances. The inhibitor from leech[49] (see Table 2) supports this hypothesis. However, before it can be confirmed, many more inhibitors from sources phylogenetically located between bacteria and mammals must be sequenced, and we plan a crystal structure analysis of a complex of PSTI with trypsin.

Origin of proteinase specificity

SSI strongly inhibits subtilisin BPN′ but only weakly inhibits α-chymotrypsin and trypsin[11,12]. BPTI and STI, on the other hand, strongly inhibit mammalian serine proteinases of the trypsin family but do not inhibit subtilisin BPN′ (ref. 1). Using the same principle as that used for preparing Fig. 3, a hypothetical complex of subtilisin with BPTI or STI can be generated. In the case of BPTI, there are serious overlaps of atoms around Cys-38′ of BPTI and around Gly-100 of subtilisin. Inspection of the model shows that these cannot be avoided by adjusting the relative orientation of the two molecules. The same situation exists for STI, where the overlaps are between atoms around His-71′ of STI and Ala-99 of subtilisin. The reason that BPTI and STI do not inhibit subtilisin BPN′ is simply due to the steric hindrance of atoms not belonging to the reactive site. In a complex of SSI with trypsin, on the other hand, there is no such obvious steric hindrance. The reason for weaker inhibition of trypsin[11] must lie in the reactive site itself. It should first be noted that P_1 of BPTI (Lys-15′) and STI (Arg-63′) form a strong ion pair with a carboxyl group (Asp-189) of trypsin[3,6]. For SSI having Met-73′ as P_1, the carboxyl group would constitute an isolated minus charge in the SSI · trypsin complex. In subtilisin BPN′, there is no charged group in the S_1 crevice[43], and the fit of the methionyl side chain seems ideal. Plasminostreptin with lysine as P_1 moderately inhibits both trypsin and subtilisin BPN′ ($K_\mathrm{I} \simeq 10^{-8}$M)[39]. Apart from P_1, we note that P_2' is arginine in both BPTI and STI. The guanidium ion has no complementary charge in the corresponding crevice of trypsin and, in this sense, tyrosine (in SSI) or phenylalanine (in plasminostreptin) might be more favourable. The P_2 residue has a bulky group in both BPTI (Cys-14′) and STI (Tyr-62′), but has only a moderately bulky group in SSI (Pro-72′) and plasminostreptin (Thr-68′). Recently, Fujiwara *et al.*[50] estimated K_I for the SSI · thiolsubtilisin complex to be 1.3×10^{-5} M at *p*H 7.0. The finding that the introduction of an atom with van der Waals radius larger only by 0.45 Å results in dramatic weakening of the association, suggests that the strong affinity between SSI and subtilisin BPN′ is highly dependent on the precise geometrical fit between the two molecules.

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The *E. coli* gene encoding heat stable toxin is a bacterial transposon flanked by inverted repeats of IS*1*

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Restriction endonuclease subclones of the Escherichia coli gene encoding the heat stable (ST) toxin exhibit a stem and loop structure similar to those seen in many procaryotic transposons. An EcoRI DNA fragment encoding tetracycline (Tc) resistance but no transposition functions was spliced into the ST gene in one of these subclones. By monitoring Tc^r, we were able to show that the ST gene transposes. Restriction and DNA sequence data strongly suggest that the ST transposon, Tn1681, is flanked by inverted repeats of IS1.

ENTEROTOXIGENIC *Escherichia coli* have been implicated in diarrhoeal disease of pigs, cattle, sheep and man[1–5]. Toxigenic strains of *E. coli* elaborate two types of toxins, one heat labile (LT) and the other heat stable (ST)[6,7]. Like cholera toxin, LT stimulates adenyl cyclase; moreover it is immunologically cross reactive with cholera antitoxin[8,9]. LT activity is assayed using either Chinese hamster ovary cells (CHO)[8] orY-1 adrenal mouse cells[10]. ST is small (less than 5,000 MW)[11,12], is nonimmunogenic and probably exerts its toxic effects via stimulation of guanylate cyclase[13,14]. ST activity is assayed in suckling mice[15]. The genetic determinants for both toxins are plasmid borne and can occur together on the same plasmid or separately[16,17]. We have reported the cloning of the gene which encodes the LT toxin as a DNA fragment of 3.0 kilobases[18] and the gene which encodes the ST toxin as a fragment of 8.9 kilobases[19]. We report here the subcloning of the ST determinant into restriction fragments ranging in size from 3.4 to 0.4 kilobases. Detailed study of one of these subclones revealed that the ST gene resides on a transposon flanked by inverted repeats of IS*1*.

Subcloning the ST gene

The construction of a plasmid hybrid (ESF3001) consisting of an 8.9 kilobase *Eco*RI fragment encoding the ST toxin cloned into RSF2124 (ref. 19) has been described. Cleavage of this fragment with *Bam*HI results in two fragments of approximately 5.6 and 3.4 kilobases (Fig. 1*a*). These two *Bam*HI–*Eco*RI fragments were cloned separately in pBR322 which had been cleaved with *Bam*HI and *Eco*RI. Removal of the internal *Bam*HI–*Eco*RI fragment from pBR322 results in a tetracycline sensitive (Tc^s) phenotype since the *Bam*HI site is within the Tc resistance gene[20]. Following transformation into *E. coli* RR1 (refs 20, 21), clones were selected for resistance to ampicillin (Ap) and then tested for sensitivity to Tc. The restriction pattern of plasmids from six clones exhibiting the Ap^r Tc^s phenotype were examined and found to contain either the 5.6 or 3.4 kilobase insert. Only the clones containing the 3.4 kilobase insert produced ST. One such clone was used for further study and was designated pMS2.

Digestion of the pMS2 insert with *Pst*I results in three fragments of 1.7, 1.4 and 0.3 kilobases (Fig. 1*a*). The central 1.7 kilobase *Pst*I fragment (Fig. 1*b*) was cloned in the *Pst*I site of pBR322. The single *Pst*I site within pBR322 occurs in a region encoding Ap resistance[20]. Transformants were therefore selected for Tc^r and checked for sensitivity to Ap. Tc^r Ap^s clones contained the 1.7 kilobase insert and produced ST. One such clone was used for further study and was designated pMS3.

Characterisation of the ST clones

Examination of single-stranded DNA from the ESF3001 plasmid which contains the *Eco*RI ST$^+$ fragment in the electron microscope[22] reveals the presence of a prominent inverted repeat (double-stranded segment) flanking a shorter single-stranded region (Fig. 2*a*). Measurement of this double-stranded segment relative to native relaxed pSC101 DNA (9.4 kilobase) included in the same grid places the size of the inverted repeat at 760 base pairs. Examination of the *Bam*HI–*Eco*RI and the *Pst*I fragments shows that the inverted repeat is present in these inserts (Fig. 2*b*, *c*). The *Pst*I sites are apparently within the inverted repeat since the double-stranded portion of the single stranded *Pst*I fragment is shorter than the original length by approximately 180 base pairs.

Figure 1*b* shows a restriction map of the pMS3 insert. Since the *Pst*I fragment has an inverted repeat (IR), restriction sites within the IR would be symmetrically arranged and digestion with these restriction enzymes would yield two DNA fragments of identical molecular weight. Digestion of the *Pst*I fragment with *Alu*I yields fragments of 392 base pairs (two fragments), 102 base pairs (two fragments), 350 and 290 base pairs, when calibrated with *Hin*cII fragments of ΦX174 RFI DNA[23]. The *Alu*I sites giving rise to the largest and the smallest fragments would therefore lie within the IR.

Many antibiotic resistance determinants are known to be transposable; they lie in discrete regions of DNA bounded by inverted repeated sequences (see ref. 24 for review). The cloned fragments of DNA containing the ST gene exhibit strikingly similar morphology. By analogy to known transposable genetic determinants, the ST gene would be expected to lie within the area comprising the loop in the stem and loop structure visualised in the electron microscope. Referring to the map (Fig. 1) the site would be located within the area delineated by the two middle *Alu*I fragments. These two *Alu*I fragments were purified and cloned separately in the *Hin*dIII site of pBR322. Since DNA cleaved with *Alu*I contains duplex or 'flush' ends, the *Alu*I fragments were ligated to synthetic decameric oligodeoxynucleotides, or 'linkers', encoding the restriction site for *Hin*dIII[25]. Neither *Alu*I fragments thus cloned gave rise to an ST$^+$ phenotype. In the same experiment we also cloned the 113 base pair *Alu*I fragment. These clones were also ST$^-$.

In a separate experiment a partial *Alu*I digest of the *Pst*I fragment produced a double *Alu*I fragment consisting of the 350 and 290 base pair fragments. This *Alu*I double fragment was subsequently purified and cloned into pBR322 again using the *Hin*dIII linkers. Clones containing this 640 base pair fragment were ST$^+$. It thus seems that the middle *Alu*I site is within an

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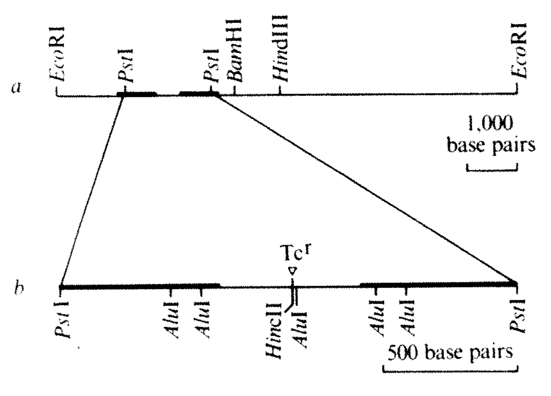

Fig. 1 Restriction maps of *a*, the 8.9 kilobase ESF3001 *Eco*RI insert and *b*, the 1.7 kilobase pMS3 *Pst*I insert. Restriction enzyme analyses of DNA fragments were performed in conditions recommended by the suppliers. *Alu*I, *Bam*HI and *Hin*cII were obtained from New England Biolabs; *Hin*dIII from Boerhinger-Mannheim. *Pst*I and *Eco*RI were purified in the laboratory according to the procedure of Greene[42]. DNA fragments were electrophoresed in 0.8% agarose in Tris-borate buffer[41], with *Eco*RI fragments of λ phage as molecular markers; or in 7% acrylamide in Tris-borate buffer, with *Hin*cII fragments of ΦX174 RFI DNA as markers[23]. *Alu*I sites were not mapped beyond the 1.7 kilobase *Pst*I fragment.

area essential for ST expression. Plasmids containing this fragment are termed pMS6.

Restriction analysis of the pMS2 insert has already shown that there is a *Hin*cII site close to the centre of the fragment within 50 base pairs of the middle *Alu*I site. To determine if this *Hin*cII site is also necessary for ST expression, we mutated the site by ligating into it the *Hin*dIII linkers. There are three *Hin*cII sites within pMS2, two of them within pBR322 (ref. 20). pMS2 plasmid DNA was digested with *Hin*cII in conditions which allowed on the average one cleavage per molecule, so that the majority of the DNA consists of unit-length linear molecules. Kinase-treated *Hin*dIII linkers were added at a molar ratio of decamer to plasmid of 25 : 1, and ligated with T4 polynucleotide ligase. The DNA was restricted with *Hin*dIII to generate cohesive termini and the preparation electrophoresed through an acrylamide gel. Unit-length linear DNA was extracted from the acrylamide, circularised with *E. coli* DNA ligase and used to transform *E. coli* RR1. Transformants were selected for Apʳ and the plasmid DNA analysed for the location of the new *Hin*dIII site. Plasmids containing the *Hin*dIII insertion at the location of the former *Hin*cII site had the ST⁻ phenotype. These plasmids were designated pMS2a.

Identification of the inverted repeats flanking the ST gene

Several lines of evidence strongly suggest that the inverted repeat flanking the ST gene sequence is IS*1*. The estimated size of the ST inverted repeat (760 base pairs) is similar to that given by Ohtsubo for IS*1* (768 base pairs) (ref. 26). In addition, Ohtsubo reported that in IS*1* there are two *Alu*I sites within the region delineated by the *Pst*I site and the right hand end of IS*1*. The sizes of these fragments are (*Pst*I–*Alu*I) 407 base pairs, (*Alu*I–*Alu*I) 113 base pairs, and (*Alu*I–end of right end) 66 base pairs; these values agree well with those estimated for the corresponding fragments of the ST inverted repeat: (*Pst*I–*Alu*I) 392 base pairs, (*Alu*I–*Alu*I) 102 base pairs and (*Alu*I–end of ST IR) 66 base pairs.

The nucleotide sequence of the pMS6 insert was determined using the Maxam and Gilbert procedure[27]; the complete sequence will be reported elsewhere. The first and last 66 bases of the sequence are inversely repeated relative to each other. The top line of Fig. 3 shows the first 70 bases of the sequence. The first and last 66 bases comprise the end of the inverted repeat proximal to the loop as seen in the electron micrographs; comparison of the sequence with the last 66 bases of the right hand end of IS*1* (ref. 27) (bottom line) show that the sequences are identical; thus, we conclude that the ST gene is flanked by inverted repeats of IS*1*.

Transposability of the ST gene

The lack of a direct method of selection for the ST gene has hampered efforts to test for its transposability. However, the restriction sites within an area essential for ST expression provided a means to test this possibility. Antibiotic resistance genes can be inserted into that region and the resistance marker used to monitor transposability and to measure the frequency of transposition. For these experiments, pMS2 was used because it contains the entire inverted repeat. Octameric linkers containing the recognition sequence for *Eco*RI was ligated into the *Hin*cII site of the *Bam*HI–*Eco*RI insert as described above. A 2.4 kilobase *Eco*RI fragment encoding resistance to Tc but specifying no transposition functions was ligated into the newly generated *Eco*RI site. This fragment was originally cloned out of pBR322 using *Eco*RI linkers[28]. As the cloning vehicle retains a portion of its own tetracycline resistance genes, the resultant hybrid was transformed into the recA strain MV12(RSF2001) to preclude recombination with the newly introduced Tcʳ genes. RSF2001 is a kanamycin resistant derivative of F (ref. 29). Transformants were selected for resistance to Tc and plasmid DNA from these clones were examined for the presence of the *Eco*RI–Tcʳ fragment. One clone containing this fragment was used for the transposition studies and is designated pMS2b.

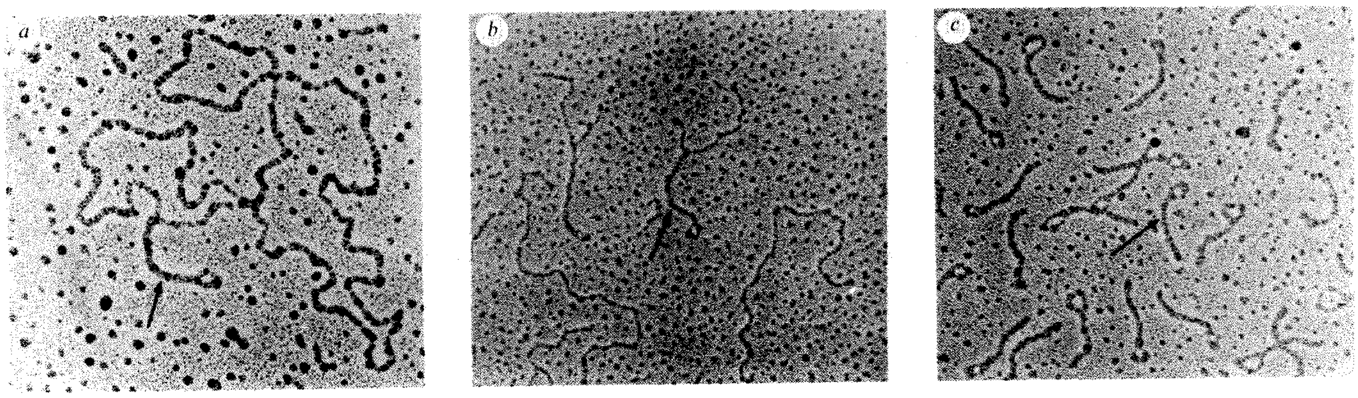

Fig. 2 Electron micrographs of single stranded DNA of *a*, ESF3001 plasmid; *b*, the 3.4 kilobase pMS2 *Bam*HI–*Eco*RI fragment; *c*, the 1.4 kilobase PMS3 *Pst*I fragment. Preparations of single stranded DNA fragments for microscopy were obtained by the procedure of Davis[22], and were examined using a JEOL 100B or a Philips 301. Micrographs in (*b*) and (*c*) were taken at the same (28,000) magnification.

```
ST IX           CTGCATGACAAAGTCATCGGGCATTATCTGAACATAAAACACTATCAATAAGTTGGAGTCATTACCTATA ...
IS1  ..ATCGGTGGAGCTGCATGACAAAGTCATCGGGCATTATCTGAACATAAAACACTATCAATAAGTTGGAGTCATTACC
```

Fig. 3 Nucleotide sequence of (top line) the first 70 bases of the pMS6 insert encoding ST, and (bottom line) the last 76 bases of the right hand end of IS*1* (as published by Ohtsubo[26]). The nucleotide sequence of the pMS6 insert was determined as described by Maxam and Gilbert[27]. DNA fragments ^{32}P-labelled at one 5′ terminus were obtained either by cleavage of the labelled fragment with a restriction enzyme or by strand separation[27].

A method for the detection of transposition of an antibiotic resistance gene from a ColE1 type plasmid has been described previously[29]. ColE1 has a strict dependence on the polI function of *E. coli*[30] while F-type plasmids do not. The strain MV12(RSF2001, pMS2b) was mated in 2.0 ml L broth with the nalidixic acid resistant (Nx^r) polI strain SF800 (ref. 29) at a donor to recipient ratio of 1:3. After 1 h of gentle shaking at 37 °C cells were plated on fresh MacConkey agar containing 20 μg ml^{-1} Nx, 5 μg ml^{-1} Tc. Since pMS2b is a ColE1 derivative, it cannot be maintained in SF800; Tc^r transconjugants arise only on transposition of the *Eco*R1–Tc^r fragment from pMS2b to the plasmid RSF2001 and the subsequent conjugal transfer of RSF2001Tc^r to SF800. Nx^{r}Tc^r clones were observed in three separate mating experiments. Six of these clones were isolated and all were able to retransfer Tc^r and Kan^r together in subsequent 1-h matings with HB101 or RR1 as recipients. The frequency of transposition of the *Eco*RI–Tc^r fragment by the ST transposon, expressed relative to the frequency of RSF2001 transfer to SF800, is $\sim 4\times10^{-6}$. In the same experiments, MV12(ESF2001,pBR322) yielded Nx^{r}Tc^r transconjugants in frequencies of $<0.01\times10^{-6}$ when crossed with SF800.

We examined 20 of these Tc^{r}Kan^r exconjugant clones from the MV12(RSF2001,pMS2b) × W3110 matings and found them to be also Ap^r and colicin immune indicating that the RSF2001 recipient plasmid contains the ColE1 portion of the pMS2b genome in addition to the ST gene. This event is IS*1* mediated and is recA independent. These observations are analogous to those of Jeffrey Miller (person communication) on Tn9, a transposon flanked by direct repeats of IS*1* (ref. 31). Using the same detection system he observes that transposition of Tn9 from a ColE1 derivative onto F results in plasmid cointegrates in which two complete copies of Tn9 have inserted into F as direct repeats flanking ColE1. Our preliminary results suggest the same sequence organisation in the recipient plasmid. In view of the observation that the transconjugants are in fact plasmid cointegrates it can be argued that the ST gene is not a true transposon. However, in rec$^+$ conditions the flanking direct repeats of the ST sequences recombine, leaving only a single copy of the ST transposon on RSF2001. Thus the end product is identical to a transposition event and it would be difficult to argue that transposition does not normally occur with such a transitory intermediate. In fact, transposition of certain mutants of Tn3 onto RSF2001 results in cointegrate plasmids with an identical sequence arrangement (Tn3–ColE1–Tn3) (ref. 32). Perhaps we are observing in these Tn3 mutants what would normally be an intermediate in the transposition process.

Discussion

Many plasmid mediated bacterial genetic determinants are known to be on transposons. They were first characterised in genes specifying antibiotic resistance in *E. coli* and *Salmonella* (see ref. 24 for review). More recently, transposons have been described in *Pseudomonas* specifying enzymes involved in resistance to mercuric ions[33] and in the degradation of toluenes and xylenes[34], and in *Yersinia* specifying enzymes for the utilisation of lactose[35]. To our knowledge the ST gene is the first pathogenic determinant shown to be on a transposon.

Most transposons are flanked by repeated sequences whose identities have yet to be established. Tn10 is reported to be flanked by inverted repeats of IS*3* (ref. 31) while in the case of Tn9 the chloramphenicol resistance (Cm^r) genes are flanked by direct repeats of IS*1* (ref. 36). Direct repeats of IS*1* have also been found on several R factors of the fi$^+$ class bracketing the resistance determinants (see ref. 37 for review). The ST transposon is the first instance in which the ends are flanked by inverted repeats of IS*1*. That IS*1* can occur in both orientations suggests that the directionality of IS*1* is not an important factor in transposition. On the other hand, Ohtsubo has reported the presence of short terminal inverted repeats within the IS*1* sequence itself; thus regardless of the orientation of the two IS*1*, the intervening genes still would be flanked by inverted repeats. And it is these internal inverted repeats which may play an essential role in transposition.

Transposable elements known to date confer easily detectable phenotypes. This, in fact, is how their transposability was first observed. Many other genetic determinants are not as amenable to such analysis because, like ST, their detection is difficult. Splicing an antibiotic resistance marker into such a gene to monitor transposition is a useful general strategy in such cases. The transposability of the ST gene accounts for the heterogeneity of the ST plasmids, which differ in their molecular size, %G+C content and incompatibility grouping[38]. A *Pst*I fragment of similar molecular weight encoding the ST toxin has been cloned from an *E. coli* enteropathogenic for pigs (N. Harford, personal communication). It would not be surprising then if the ST toxins elaborated by *E. coli* strains are closely related if not identical proteins.

Simple acquisition of an ST plasmid does not render an *E. coli* pathogenic. Besides producing ST, a strain must also elaborate an adherence or colonisation factor (CF) before it can become fully pathogenic[1,2,39,40]. Such factors are plasmid encoded and are cell and animal specific. The colonisation factor is expressed as filamentous cell surface proteins which enable the organism to adhere to the mucosa of the upper bowels of the animals, an area normally devoid of such organisms. In addition, diarrhoeagenic *E. coli* isolated from pigs elaborate a CF different from those isolated from calves or man. In the course of association between an ST and a CF plasmid within a pathogenic *E. coli* one would expect the eventual acquisition of the ST gene by a CF plasmid. Indeed such a case has been found. A plasmid encoding the human colonisation factor has been shown to harbour the ST gene as well (P. Shipley, personal communication).

Other pathogenic determinants such as the heat labile (LT) toxin and the K antigens may also reside on transposons. Silva *et al.* have reported the presence of inverted repeats bounding an area known to contain genes for both the ST and the LT in a plasmid isolate of an enteropathogenic *E. coli*[41]. Transposition has proved to be an important mechanism in the dissemination of antibiotic resistance; it may ultimately prove to be as common a mechanism as conjugation and phage conversion by which pathogenic determinants are acquired by bacterial genomes.

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Death throes of massive stars

MASSIVE stars undergo violent deaths, forming the type II supernovae believed to result from gravitational collapse of the stellar cores. Such an event may be recognised by the optical outburst which occurs when the thermal and kinetic energy generated by the collapse propagates to the stellar photosphere. This occurs ~5–20 d after the collapse[1]. However, the optical data are incomplete in the sense that supernovae are invariably discovered at or shortly after peak optical intensity so only the decline from maximum brightness has been recorded in any detail. In addition, various theoretical models suggest that the optical radiation will be preceded by bursts of other forms of energy (neutrinos, gravitational waves, γ and X rays). Detection of these bursts, as well as of the early part of the optical light curve, would be greatly facilitated if the occurrence of a supernova could be predicted on the basis of the pre-supernova behaviour of the star. We show here that neutrino emission in the final stages of nuclear burning (as originally proposed by Morrison[2]) could, in principle, provide such a warning, though the current practical limitations are severe.

Our results are based on a study of the final stages of evolution of a $15M_\odot$ star (details will be published elsewhere). The evolution through hydrogen, helium, and carbon burning was computed with a hydrostatic stellar evolution code[3]. The subsequent stages (neon, oxygen, and silicon burning) were followed with an implicit hydrodynamic code[4]. The nuclear reaction network included the species ^{1}H, ^{4}He, ^{12}C, ^{16}O, ^{20}Ne, ^{24}Mg, ^{28}Si, ^{32}S, ^{36}Ar, and ^{56}Fe. Neutrino emission was computed according to the rates of Beaudet *et al.*[5] which include the pair-, photo-, and plasma-neutrino processes. The Weinberg–Salam model for weak interactions suggests that these rates should be revised upwards[6], but experimental evidence[7] indicates that the correction is less than a factor of 2. All of these processes produce equal numbers of (electron) neutrinos and antineutrinos.

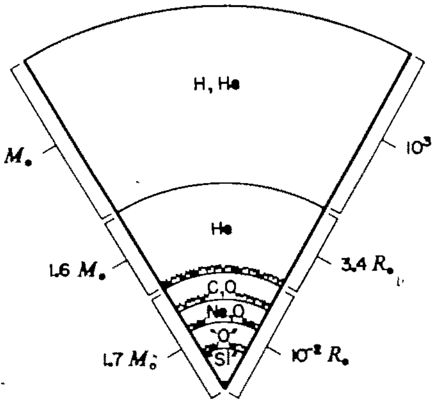

Fig. 1 Cross-section (not to scale) of the star at the beginning of the silicon-burning phase. Jagged lines indicate active burning regions. The composition of the O and Si zones consist of a number of species; 95% of the mass in the inner (1.7 $M_\odot$) region is contained in the Si zone.

The structure of the star at the start of the period of intense neutrino emission is shown in Fig. 1. This represents the beginning of the core-silicon-burning phase. The results of the calculations for the subsequent evolution are shown in Fig. 2, in which nuclear burning and neutrino energy emission rates are plotted against time. Average neutrino energies at representative times are also indicated. As the dominant neutrino emission process is the destruction of electron–positron pairs, the neutrino energies include the 0.511 MeV rest-mass energy of the annihilated particles. The first (broad) peak in neutrino emission represents core silicon burning, which occurs several months before collapse, and subsequent (sharp) peaks are due to increasingly violent shell flashes. The last major burst corresponds to a silicon flash ~4 d before collapse. We should emphasise that, although the nuclear energy generation rate varies by 7 orders of magnitude during this period, no detectable variations in the surface photon luminosity occur. To an optical observer, the star appears as a perfectly normal red supergiant and gives no warning of the impending explosion.

To examine the detectability of the predicted fluxes, the background levels over which the stellar neutrinos (or antineutrinos) must dominate should be considered. For this purpose we choose the last peak, at which time the neutrino (plus antineutrino) luminosity is $6\times10^{11}L_\odot$ and the average energy per particle is 0.83 MeV. For the neutrinos, the main background is the Sun. Optimal discrimination against solar neutrinos is afforded by a detector which is insensitive to the copious p–p neutrinos, such as the ^{37}Cl experiment of Davis[8]. The condition that the capture rate for stellar neutrinos be greater than for solar neutrinos is

$$\sigma_\nu F_{\nu *} > \Sigma\sigma_\odot F_{\nu\odot} = 1.6\times10^{-36}\ \mathrm{s}^{-1} \qquad (1)$$

where we have used the measured value for the solar neutrino capture rate[9]. With $\sigma_\nu = 2.4\times10^{-46}$ cm^2, we find that the pre-supernova object would have to be within 34 pc to be detected. Obviously this is unacceptable. Note that Bahcall[10] has estimated that the neutrino burst due to core collapse would be detectable at a distance of 4.5 kpc. However, radiochemical detectors are generally not very suitable for supernova prediction as they involve long integration times and give no directional information.

The situation is more favourable for the antineutrinos, where the solar background is negligible and the main background is

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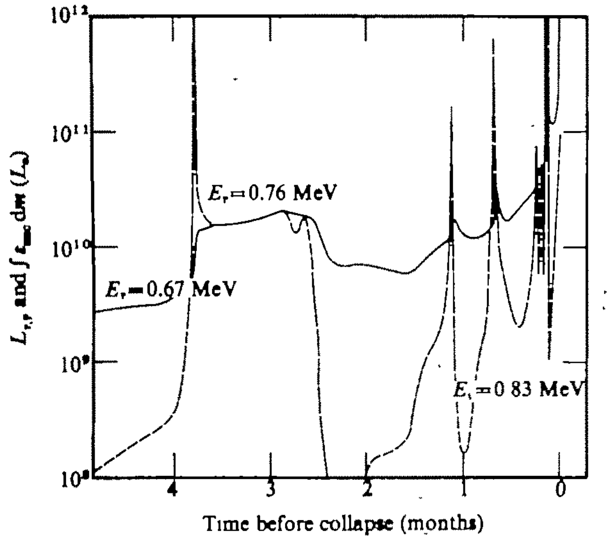

Fig. 2 Total energy emission due to neutrino pairs (solid line) and energy generation by nuclear reactions (dashed line) as functions of time. Average neutrino energies are indicated at various points. The $E_\nu = 0.83$ MeV notation refers specifically to the last silicon flash.

due to radioactive decays in the Earth's crust. The flux of terrestrial antineutrinos is not well known, but it is estimated to be $F_{\bar{\nu}\oplus} \sim 3.5 \times 10^6$ cm^{-2} s^{-1}, if we eliminate the decays with peak energies below 0.5 MeV[11]. As the remaining decays yield energies comparable to the expected stellar antineutrino energies, we can directly compare fluxes, without referring to cross-sections for a particular detector. The condition $F_{\bar{\nu}} > F_{\bar{\nu}\oplus}$ now leads to a distance limit of 1.5 kpc. With an expected event rate of ~0.1 supernova yr^{-1} in the entire Galaxy, this is not very encouraging. If we wish to monitor the entire Galaxy, a space experiment would be necessary. For a satellite in geosynchronous orbit, the terrestrial antineutrino background would be negligible, although the technical difficulties involved in designing a suitably sensitive detector should not be underestimated.

Antineutrinos emitted by massive stars in the final stages of nuclear burning could then be used to predict supernovae, though major improvements in detection techniques would be required. Note that the results presented above apply to a particular stellar mass (15 $M_{\odot}$) and that other masses might give substantially higher fluxes.

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An ultraviolet spectrum of the high redshift quasar Q2204 – 408

AN ultraviolet spectrum of the radio-quiet quasar Q2204 – 408, redshift $z = 3.18$ (ref. 1), detected with the International Ultraviolet Explorer (IUE) is reported here. The object was selected for observation because of its high Lyman-α flux and the absence of a cut-off at the Lyman limit. The spectrum was obtained on 9 August 1978 with the short wavelength spectrograph which covers the range 1,150–1,950 Å (ref. 2). A finder field for Q2204 – 408 has been given by Osmer and Smith[1] and as the quasar is too faint ($m_v = 17.5$) to be detected by the fine error sensor of IUE, a blind off-set technique was used to image the quasar in the large (10 × 20 arc s) aperture of the short wavelength spectrograph. A very faint continuous spectrum of average signal ~5 DN above a background of ~60 DN was detected after an exposure of 350 min. (DN stands for data number and 256 DN gives full scale.) An emission feature is also probably present at 1,270 Å (304 Å in the rest frame of the source).

A computer program was developed to track the spectrum in both the raw and the photometrically corrected images. The data, in a strip 18 pixels wide and centred on the spectrum, were extracted without rebinning and only wavelengths longer than the night-sky Lyman α line have been considered. The data in the photometrically corrected image were compared with those in the raw image, and a visual examination made of the photowrite of the raw image so as to identify and remove signals caused by cosmic ray events and other noise spikes. The data in the central 6-pixel wide strip were combined and a background correction made by subtracting the smoothed signal on either side, thus giving the net spectrum. The signals were converted to absolute fluxes with the most recent[3] absolute efficiency of the short wavelength spectrograph. A scaling factor was used to convert the flux value obtained with the data extracting procedure used here to that which would be obtained with the extraction procedure used to derive the absolute efficiency of the spectrograph. This was determined to be ~1.2 by reducing the spectrum of the quasar MKN205 (also observed by us) with the two extracting procedures, and comparing the outputs.

The spectrum of Q2204 – 408 was averaged in 100 Å bands and the resulting fluxes and central wavelengths are given in Table 1. The r.m.s. errors are also given and the increase at the longer wavelengths is due to a high local background that was accumulated during the long exposure. The data between 1,400 and 1,500 Å have not been used, as they lie in a sensitivity minimum of the instrument (caused by the photocathode in the camera) where the signal becomes too low to be significant. Because of the large errors, the UV data have been combined to give the weighted mean listed in Table 1, where the error has been increased to allow for the uncertainty of ~10% in the absolute calibration[3]. The UV flux can now be compared with the visible flux measurement of Osmer and Smith[1] also given in the Table.

Table 1 Fluxes and central wavelengths for Q2204 – 408

Wavelength (Å) Rest frame of observer	Flux (10^{-16} erg cm^{-2} s^{-1} Å^{-1})	Wavelength (Å) Rest frame of source
1,350	4.2 ± 1.0	323
1,650	10.4 ± 1.4	395
1,750	9.9 ± 4.9	419
1,850	5.5 ± 3.4	442
1,480*	6.3 ± 1.0	354
6,165†	2.9	1,475

*UV weighted mean.
†From Osmer and Smith[1].

As usual, we assume a continuum flux distribution in frequency terms of the form $f(\nu)\alpha\nu^{-\alpha}$ and from the UV and visible data derive a value for the spectral index $\alpha = 1.5 \pm 0.3$. This is in the range found for quasars from optical and radio data and shows that the non-thermal continuum extends to wavelengths as short as ~300 Å in the rest frame of the source. It also confirms the great transparency of the intergalactic medium which has been well established by several workers (see, for example, refs 4, 5). A difference here is that the observed flux has been subjected to the full absorption characteristics of neutral hydrogen, that is, in the Lyman continuum and Lyman series, and this occurs in a local epoch ($z < 1$). Additionally, the flux will suffer absorption by any intergalactic neutral helium in the early epoch $z \sim 2$–3.

The extracted spectrum shows an emission feature near 1,270 Å which is probably real. Its wavelength is uncertain because the only internal standard is provided by the night-sky Lyman α line whose signal is saturated. However, the wavelength is clearly close to 304 Å in the rest frame of the quasar and the feature is likely to be the redshifted HeII resonance line. Its total intensity is estimated as $\sim 2 \times 10^{-14}$ erg cm^{-2} s^{-1} which can be compared with the intensity of (Lyman α + NV) = 11×10^{-14} erg cm^{-2} s^{-1} given by Osmer and Smith[1]. Further observations are being made which should confirm the reality of the feature.

The observations were carried out during UK time awarded by the SRC at the ESA Ground Station near Madrid. We acknowledge the support of Dr A. Heck and Mr F. Pevar during observations, and of Dr N. Argue in measuring off-sets.

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The August 1972 solar proton event and the atmospheric ozone layer

OXIDES of nitrogen are generally accepted to be important for regulating the atmospheric ozone content through catalytic reactions first described by Crutzen[1] and Johnston[2]. In the stratosphere, nitric oxide is produced naturally by the reaction of nitrous oxide, emanating from the biosphere, with excited oxygen atoms generated by ozone photolysis at wavelengths shorter than about 310 nm. As Crutzen *et al.*[3] have pointed out, large amounts of nitric oxide may also be produced by high-energy solar protons, mainly at geomagnetic latitudes greater than 60°, and altitudes above about 35 km. The ozone measurements reported here made during an intense solar proton event in August 1972 suggest that NO production rates due to ionising particles are higher than those calculated by Crutzen *et al.*

This process of NO production is likely to occur in several steps: nitrogen molecules are ionised and dissociated by secondary electrons with energies in the range of tens to hundreds of electron volts. The subsequent reaction $N + O_2 \rightarrow NO + O$ is slow and temperature dependent for ground N (^{4}S) atoms, but not for electronically excited N (^{2}D or ^{2}P) atoms. As the electronic states of the N atoms were not known, Crutzen *et al.*, in their computation of NO production rates for three major solar proton events, considered two extreme situations with either complete or no production of N atoms in the excited states.

Recent rocket measurements of mesospheric and thermospheric nitric oxide concentrations revealed strong enhancements during auroral particle precipitation events (ref. 4 and F. Arnold, personal communication). From these measurements, and simultaneous measurements of ionisation rates, it is inferred that the NO-production rate is about 2–2.5 times the ionisation rate, while Crutzen *et al.* base their estimates on 1.5 mol per ion pair, as given by Winters[5]. These values imply that most of the N-atoms resulting from electron impact dissociation of N_2 are probably in an electronically excited state[4].

On 4 August 1972 a solar proton event occurred that was the most intense of any recorded within the past 25 yr. Ozone measurements made with the BUV experiment aboard the Nimbus 4 satellite during that period were the first ever to show unambiguously a decrease in stratospheric ozone due to a solar proton event[6]. Figure 1 shows total ozone amounts above the 4 mbar level, for three latitude belts, as published by Heath *et al.*[6]. Zonally averaged daily values are plotted with the respective error bars for the months of July and August 1972. In the polar region, between 75° and 80° N, the event produced an abrupt decrease in ozone above 4 mbar, of about 0.002 atm cm or 16%, which apparently persisted throughout the month of August. At latitudes between 55 and 65° N a very sharp drop was noticed 2 d after the event followed by a slow decrease of 5% within 8 d, and a gradual 'recovery' thereafter. Heath *et al.* interpreted this behaviour as being due to the advection of modified air from the source region, which is symmetric in geomagnetic coordinates by geographically orientated zonal motions, with the succeeding 'recovery' being similar to the seasonal changes found in other years in this latitude range. The

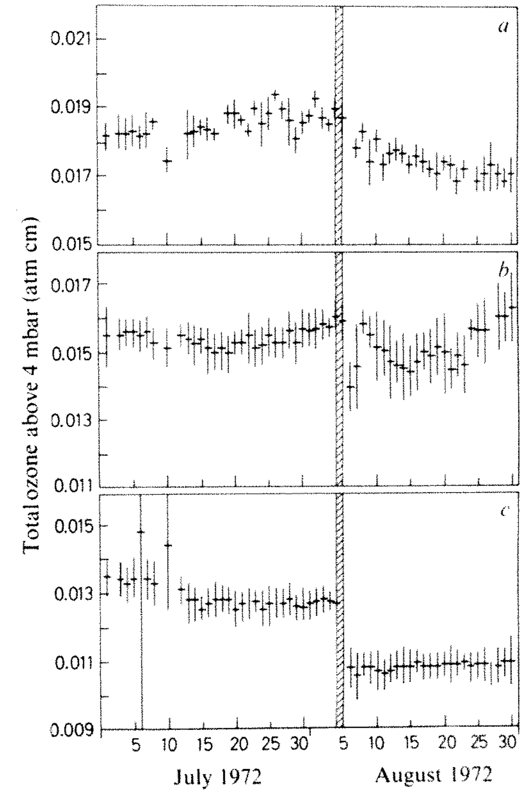

Fig. 1 Zonally averaged total O_3, above the 4-mbar pressure surface for equatorial (*a*), middle (*b*), and high latitudes (*c*) during July and August 1972. The solar proton event occurred on 4 August. After Heath *et al.*[6].

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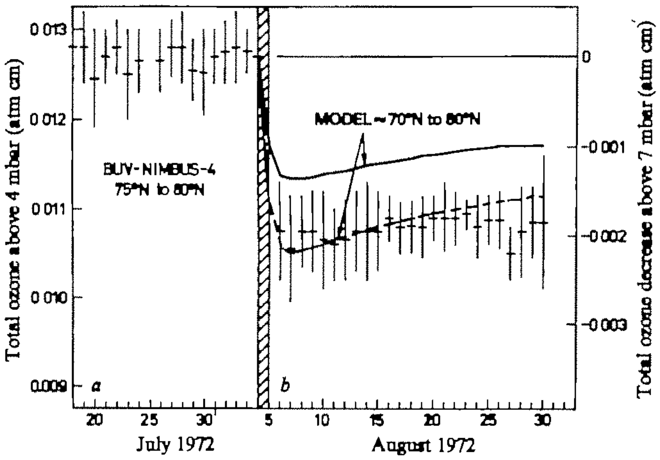

Fig. 2 Comparison of BUV–NIMBUS-4 ozone data (*a*) with model prediction for high latitudes (*b*). The model results are plotted as relative depletions with respect to the 'undisturbed' level on 4 August, before the event occurred. The solid line bases on NO production rates as given by Crutzen *et al.*[3]. The dashed line includes Arnold's modification[4].

ozone measured in the tropics are, although different from those observed in earlier years, not related uniquely to the proton event. According to Heath *et al.* the slow ozone increase during July and the steeper decline in August are likely to be partly due to an abnormal temperature decrease in July associated with a Southern Hemisphere stratospheric warming, followed by a recovery in August.

As Crutzen *et al.*[3] pointed out, studies of the ozone distribution in the stratosphere in periods following certain proton events, may be important for validating numerical models of photochemistry and transport processes in the stratosphere. We have performed such modelling experiments using the Oxford zonally averaged, two-dimensional model, described in detail by Harwood and Pyle[7]. This time-dependent model extends from the South Pole to the North Pole, with 19 latitude belts of slightly less than 10° width, and from the Earth's surface to about 80 km altitude, with a height increment of about 3.5 km (0.5 pressure scale heights). Eddy fluxes of heat and matter are treated using large-scale diffusion coefficients, while the momentum fluxes are specified from temperature measurements made mainly by the selective chopper radiometer on the Nimbus 5 spacecraft. Radiative processes, such as the absorption of solar radiation by ozone and the radiative cooling due to the CO_2 and O_3 emissions, are included in the model. The photochemical scheme, which was specified in the lowest 17 layers corresponding to the lowest 60 km of the atmosphere, comprises the most important photochemical reactions determining the concentrations of oxygen, nitrogen, and hydrogen compounds. Continuity equations are written for the following species or groups of species: 0, O(^{1}D), O_3; N, NO, NO_2; HNO_3; H_2O_2: H, OH, HO_2 (ref. 8). For the latter group the photochemical time scales are short and dynamical processes are ignored.

The solar proton simulation experiments were performed after the model had reached a quasi steady state thus ensuring that all trace gas variations were merely due to seasonal changes. On 4 August, when the event occurred, a sudden injection of NO was introduced in the model. In a first experiment we used NO production rates for the August 1972 event as calculated by Crutzen *et al.*[3] for excited N atoms. (In fact, the differences of NO production rates between ground-state and excited N atoms only become important for altitudes above 50 km, whereas the highest ozone depletion rates through this effect are found below that altitude. Additional test runs showed that the ozone depletions for both cases differ by 7%.) A second experiment was run with the same set of NO injection rates, but multiplied by a factor of 1.7 as suggested by Arnold's findings. For both cases the solar proton-produced NO was introduced at geographical latitudes greater than 60°. It is inherent in our zonally averaged approach that geomagnetic and geographic latitudes are assumed to be coincident. The results of these experiments are given in Figs 2 and 3 for polar and mean latitudes, respectively. For comparison with the BUV data as taken from Fig. 1 (scale on *a*) the model results are plotted as relative depletions with respect to the undisturbed conditions immediately before the proton event occurred (scale on *b*). Total ozone was integrated above the 6.8 mbar level of the model which yielded the best match with the total ozone amounts indicated, obtained above 4 mbar by the BUV experiment.

For the polar latitudes (Fig. 2) the model results fit the observations reasonably well when the NO production data by Crutzen *et al.*[3] are increased by 1.7 as discussed earlier (dashed line). Without this modification, as shown by the solid line, the predicted ozone drop due to the proton event is too small. The same holds for the model results for the 55 to 65° latitude belt shown in Fig. 3. As this latitude region is covered by two adjacent latitude belts in the model, that is ~50 to 60° and ~60 to 70°, predictions of ozone depletion for both are plotted separately. Superimposing the two dashed lines, corresponding to modified NO production rates, there is, within the accuracy indicated by the error bars, good agreement with the BUV observations, before the 'recovery phase' which starts after about 20 August. If the interpretation offered by Heath *et al.* for the mid-latitude changes is correct, we should not expect a zonally averaged model to reproduce all the details of the effect. Furthermore, one should bear in mind that the ozone shows natural variations illustrated best by those observed in the tropical region (Fig. 1*a*) where the solar proton event did not cause any measurable effect.

We have investigated the changes in the ozone budget in the model following the event. Thus we have separated the net rate of change of ozone into contributions due to the photochemical and the mean and eddy transport terms. In high latitudes, the increased photochemical destruction during the event is counteracted to some extent by the transport. For instance, between about 60° and 70°N, where the perturbations to the ozone concentration are largest, the eddy terms tend to increase the ozone while the mean motion terms, which on average remove ozone from this region, are reduced in magnitude following the event.

It is interesting that the model overestimates the ozone recovery after about 23 August, at the highest latitudes considered, but underestimates it in middle latitudes. It is possible that the meteorological conditions prevailing at this particular

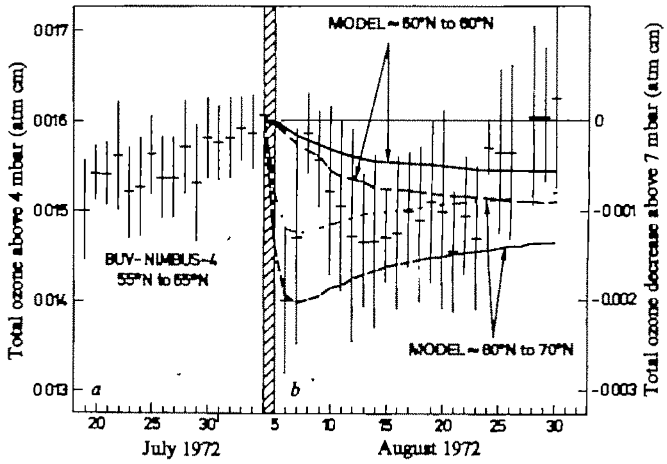

Fig. 3 Comparison of BUV–NIMBUS-4 ozone data (*a*) with model prediction for midlatitudes (*b*). The 55 to 65° N latitude range of the BUV data is somewhat between the latitude belts 50 to 60° N and 60 to 70 °N of the model. The solid line and the pointed line correspond to NO production rates given by Crutzen *et al.*[3] for the two latitude belts, respectively. The dashed lines include Arnold's modification[4].

time were such that there was a transport of ozone from high to middle latitudes, larger than calculated in the model. Clearly a model using monthly diffusion coefficients and momentum fluxes cannot be expected to reproduce dynamical changes on a synoptic time-scale. For this reason we do not discuss here our results for the Southern Hemisphere, where the calculated ozone changes were smaller, because the circulation, and thus the ozone distribution, would have been dominated by the aforementioned stratospheric warming. The model did predict temperature and circulation changes following the event but these were small, with temperature reductions not greater than 1% in the region of the greatest ozone changes. Using data from the Nimbus 4 selective chopper radiometer, the temperature sounding instrument built by the Department of Atmospheric Physics at the University of Oxford, we have looked for changes in temperature following the event. The top channel of the instrument provides information on the temperature averaged over about a 10 km thick slab in the upper stratosphere. The radiance data from this channel showed no significant changes associated with the event. It would appear that the feedback between the ozone concentration and temperature was not particularly important in this case. Indeed, our preliminary runs in which this feedback was suppressed, produced substantially the same results.

The results suggest that the NO production rates due to ionising particles are possibly higher than those originally calculated by Crutzen *et al.*[3] although the theoretical study of Porter, Jackman and Green[9] indicates even smaller rates. This work stresses the importance of the study of such natural phenomena for our understanding of atmospheric photochemistry and dynamics. It would be a major advance if, during one of the next major solar proton events, other related minor constituents could be measured. In this respect, satellite experiments such as those on Nimbus G, gain additional importance.

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Energetic protons near the plasma sheet boundary

IT has long been evident that energetic particles are produced in the Earth's magnetotail in association with magnetospheric substorms[1-6]. These particles may be important in producing ionospheric effects such as auroral zone absorption and they also lead to the injection of energetic particles into the interplanetary medium. We provide here an interpretation for the behaviour of a class of very intense, short-lived bursts of protons and other ions which appear at the edge of the plasma sheet during the expansion phase of substorms.

The characteristics of impulsive proton bursts in the magnetotail were first described by Sarris *et al.*[7] using observations of energetic proton and electron fluxes with good time resolution (~10 s) made at a geocentric distance of ~35 R_e by experiments on the spacecraft IMP 7 and 8. The bursts are observed only on the dusk side of the magnetotail, have durations of 10–30 s at a given energy (perhaps depending on the energy resolution of the detectors) and are not detected beyond ~1.8 MeV in energy. The differential proton intensities at ~300 keV can be as large as 10^5 cm^{-2} s^{-1} sr^{-1} MeV^{-1} and they are highly collimated along the magnetic field direction with anisotropies as large as 10^5:1 directed towards the tail. The most important features of the bursts is that they exhibit an inverse velocity dispersion. That is, the bursts are observed first at low energies and at higher energies some 10–20 s later, frequently when the low energy proton burst has already subsided. In general, energetic electron bursts are not observed in association with these impulsive proton bursts.

At least 12 of these bursts have been found and they have been shown by Roelof *et al.*[8] to be directly associated with the expansion phases of large geomagnetic substorms. An examination of the University of Iowa LEPEDEA spectrograms (L. A. Frank and K. L. Ackerson, personal communication) reveals that the bursts occur near the outer boundary of the plasma sheet during times when the plasma sheet disappears from the spacecraft (that is, during 'thinning'). Measurements of the magnetic field on the same spacecraft indicate that, in the case described here, the burst coincided with a transient perturbation of the magnetic field in both magnitude and direction (N. F. Ness and R. Lepping, personal communication). Observations of multiply-charged species indicate that in this event these particles behave in a similar fashion to that described above for protons (G. C. Gloeckler, personal communication).

Figure 1 shows the responses of the P1 (0.29–0.50 MeV), P2 (0.50–0.97 MeV) and P3 (0.97–1.85 MeV) channels of the

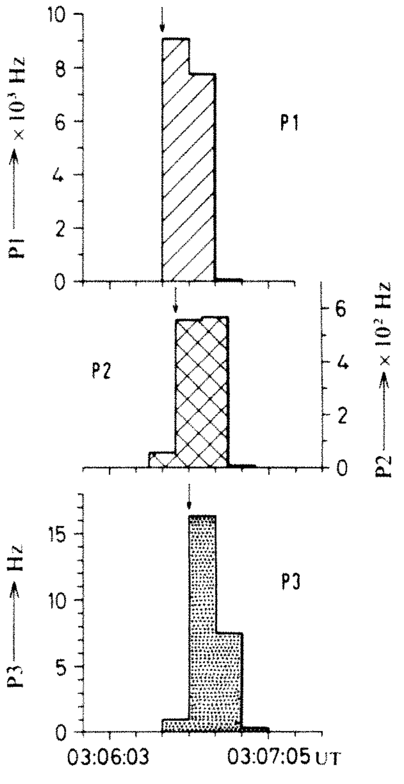

Fig. 1 Count rates of the P1 (0.29–0.50 MeV), P2 (0.50–0.97 MeV), and P3 (0.97–1.85 MeV) channels with a 10-s time resolution during the impulsive proton burst described in the text. (JHU-APL IMP 7 experiment, 27 March 1973.)

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JHU-APL IMP 7 experiment during an impulsive proton burst which took place on 27 March 1973 at about 03.06.30 UT. The spacecraft was situated in the dusk magnetotail with solar magnetospheric coordinates $x = -31.5\ R_e$, $y = 16.6\ R_e$, $z = -5.0\ R_e$. The LEPEDEA spectrograms show that the burst was associated, to within the accuracy allowed by the observations, with a plasma sheet boundary crossing in which the plasma sheet disappeared from the position of the spacecraft. The duration of the main pulse in each energy channel was ~20 s. The University of Maryland experiment on the same spacecraft, using a detector with a smaller energy window (125–160 keV per charge, $z \geq 1$), found the pulse width to be noticeably smaller, namely ≤ 5 s (G. C. Gloeckler, personal communication). It is clear from Fig. 1 that the low energy (P1) burst was observed first and that it was followed ~10 s later by the higher energy (P3) burst. The arrows on the figure mark edges of the burst where the proton intensities increase sharply by more than an order of magnitude.

There was no electron burst associated with the proton burst, although weak fluxes of electrons were present before the burst. It is possible however, that a very short-lived ($\ll 1$ s) electron burst could have occurred and escaped detection as a consequence of the ~1.3 s spin period of the spacecraft. A telescope responding to α-particles in the energy range $0.64 \leq E \leq 1.17$ MeV per nucleus indicated that no α-particles were present in this energy range. The University of Maryland experiment detected multiply-charged ions ($z \geq 2$) with an intensity about 10% of that of all particles with $z \geq 1$ and apparently having a rather sharp energy cut-off in the range 800–1,200 keV. We have estimated the possible contribution of heavy ions to the P1, P2 and P3 counting rates using the observations of magnetospheric ion bursts reported by Fan *et al.*[9] as a basis, and find that such ions are likely to make a significant but small contribution to the counting rates. However, on the basis of the measurements presently available we cannot rule out the possibility that helium and oxygen ions (perhaps singly-ionised) make a substantial contribution to the counting rates which we have attributed to protons.

The properties of the proton bursts described above are consistent with the presence of a field-aligned sheet-like distribution of energetic protons which moves rapidly past the point of observation. The particles appear to have originated at a point on the sheet lying between the position of the spacecraft and the Earth and the magnetic field lines must be open so that the particles can escape freely. There is no evidence for any north–south difference in the occurrence of the bursts so that it can be expected that similar sheets exist simultaneously on both sides of the plasma sheet. The two sheets may therefore meet at a line where the particles originate, and this may be associated with magnetic field reconnection taking place at a neutral line which is (temporarily) situated between the spacecraft and the Earth[10]. In the following we examine the whole phenomenon from this point of view.

The situation must be such that the magnetic field lines which are reconnected at the neutral line are open, 'lobe' field lines (that is, with one end connected to interplanetary space) and with the plasma concerned being part of the very low density population observed in the lobes of the magnetotail[11]. The characteristic Alfvén speed in these circumstances is very high indeed (perhaps several thousand km s^{-1}) and one can expect that, for a short time at least, reconnection takes place very rapidly with large inductive electric fields existing in the vicinity of the neutral line and voltage drops of ~1 MV. The situation is rather similar to that found in laboratory reconnection experiments where the reconnection rate is observed to suddenly increase, with a corresponding enhanced production of energetic particles, at the time when magnetic field lines carrying low plasma densities reach the neutral point[12]. Note that these electric fields are not directly observable at ionospheric levels, however, one is naturally led to associate the neutral line with a type of auroral arc which moves rapidly polewards during the expansion phase of substorms[13–15].

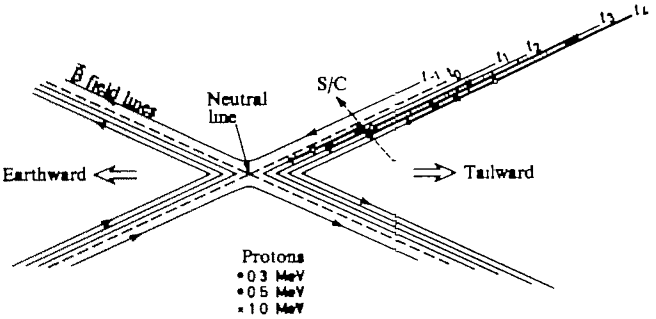

Fig. 2 Schematic diagram of the motions of magnetic field lines and the associated accelerated energetic particles resulting from magnetic reconnection. The positions of particles released from the neutral line at t_0 and at progressively later times t_1, t_2, t_3 and t_4 are shown more or less to scale, as is the motion of the spacecraft relative to the whole configuration

In this context the inverse velocity dispersion may be interpreted as follows: it is assumed that particles on a given field line are accelerated more or less simultaneously in a small region surrounding the neutral line as the field line becomes reconnected (that is at the time it reaches the neutral point). Following reconnection, the magnetic field lines carrying this population of energetic particles move tailwards and earthwards away from the neutral line and on the tailwards side, the particles stream freely away from the Earth into interplanetary space. A schematic of the motions of magnetic field lines and the energetic particles is shown in Fig. 2. Reconnection of a particular magnetic field line and the associated particle acceleration is assumed to occur at time t_0. At subsequent times (t_1, t_2, t_3, …) this magnetic field line moves away from the neutral line while the energetic particles it carries disperse along the line such that the faster particles are at any time further away from the source. During an outbound crossing of the plasma sheet boundary by a spacecraft (which is the case here, since the plasma disappears from the vicinity of the spacecraft) the magnetic field lines should appear as almost perfect 'velocity selectors' such that the lower energy particles are observed on the inner lines and the higher energy particles on the outer lines with respect to the plasma sheet. Thus particle detectors on the spacecraft could in principle detect a sequence of mono-energetic pulses with the energy increasing in time at a rate depending on the speed of the field lines and the motion of the plasma sheet boundary relative to the spacecraft. With finite energy and time resolution and a finite source size, the pulses must, of course, have finite width, but the dispersion effect will not be changed. In the present example, we estimate from the dispersion and the width of the pulses that the speed of reconnection is of the order of a few times the speed of the boundary motion, if the reconnection point is at a distance of ~20 R_e from the Earth.

How do these particles become energised? There is mounting evidence that much of the energy dissipated resistively in the reconnection process goes into the production of high energy particles[16–18]. The rather pronounced dawn–dusk asymmetry[2,7,19,20] suggests a simple cross-tail acceleration mechanism associated with magnetic field reconnection[21–23]. In this case one might expect the high energy channels to be dominated by highly charged species (for example O^{6+} and Fe^{9-13+} from the solar wind, if available) and this does not seem to be in agreement with the observations. However, if the reconnection takes place at a distance of the order of 20 R_e from the Earth it is likely that the plasma involved is of ionospheric origin and therefore singly charged. It is also possible that there could be local electrostatic acceleration in the vicinity of the neutral point, resulting from the fact that the electrons are constrained to move with the magnetic field lines, whereas the ions become 'non-magnetic' and hence the necessity for charge neutrality might require that the ions be ejected from this region. This would produce a similar energy per charge signature to the cross-tail acceleration mechanism, but the dawn–dusk asymmetry could not be easily explained. The latter seems also to be a difficulty for any stochastic mechanism as does the potentially large gyro-radii of the ions and the very high anisotropy which is produced. Finally, pulses with the reversed velocity dispersion should be observed when the plasma sheet envelopes the spacecraft during the 'thickening', which takes place at a later stage in the substorm. We expect, however, that the voltage drops prevalent at such times are much smaller than during thinning and hence that the particle bursts should be detectable only by instruments with lower energy thresholds than described here.

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Temperature dependence of atmospheric absorption in the wavelength range 8–14 μm

THE Earth's atmosphere has relatively high transparency to wavelengths between 8 and 14 μm, which makes them particularly important for communications and remote sensing. A reliable model of molecular absorption in this range is of particular value[1] as it may be used to relate atmospheric temperatures to radiant flux measurements made by artificial satellites—it seems that even in conditions when particle scattering can be neglected, absorption losses can be greater than is expected from present models. To study the remaining loss, or anomalous absorption, we have made new measurements in which particular attention was given to the temperature dependence of the anomalous component. The motivation for this was to test the hypothesis[2] that anomalous absorption could be ascribed to an equilibrium concentration of water dimers in the atmosphere. We find that this explanation cannot account for anomalous absorption in all conditions.

Measurements were made on a horizontal column of atmosphere 1.5 m above ground level and 560 m in length. The spectral observations were supplemented by laser measurements at wavelengths of 10.6 and 0.63 μm, the latter being used to quantify visibility. Standard meteorological determinations of air temperature and relative humidity were also made. A total of ~2,000 h of usable observation in various weather conditions was obtained between January and September of 1977. We have limited this discussion to conditions when the visibility was greater than 40 km so that the infrared absorption we measured would not be complicated by particle attenuation, of which the upper limit could be estimated from the visibility.

The first step in the analysis was to separate out the water monomer component which is predictable from the measured humidity and temperature. The input to these model calculations is summarised in Table 1 and a representative spectrum is shown as curve *m*–*m*′ in Fig. 1. The values of anomalous absorption were then derived by subtracting predictions from observations. Contours were formed by taking average values for each of the 15 low absorption regions (windows) shown at the top of Fig. 1 and joining up their absorption ordinates at the mid-wavelength points, the windows being chosen to exclude most of the water monomer line centres.

The attribution of anomalous absorption to water dimers rests entirely on the observed temperature dependence being in agreement with that expected from the known intermolecular binding energy of the dimer. To test the hypothesis, therefore, we used equation (1) to reduce the field observations to standard conditions of density and temperature.

$$\alpha = Cd^2 \exp(B/k\theta) \qquad (1)$$

α is an absorption coefficient in dB km^{-1}; d is the measured water vapour density; B is a temperature exponent in eV per molecule, k is Boltzmann's constant in eV per molecule per deg K; θ is the temperature in K; C is a constant containing the relevant absorption cross sections which are assumed to be independent of temperature.

The values of B were then calculated for each window wavelength and the data were also separated into low and high temperature blocks to see if the exponents were themselves dependent on temperature. The division was made at 286 K to give about equal amounts of observation in the two ranges

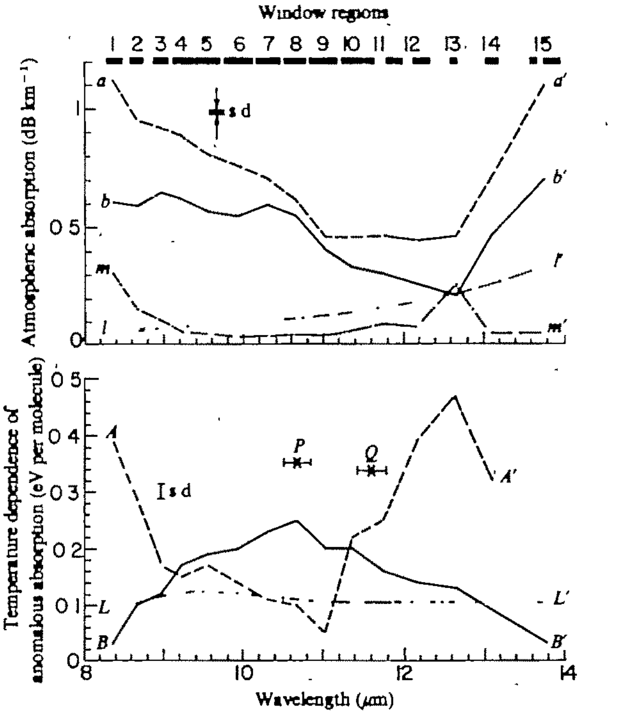

Fig. 1 Curves *a*–*a*′ and *b*–*b*′ show observed anomalous absorption values for mean temperatures of 281 and 290 K respectively, reduced to a water vapour density of 4.8 g m^{-3}. The estimated error is ±0.02 dB km^{-1}. Curves *A*–*A*′ and *B*–*B*′ are the corresponding temperature dependence values expressed as the exponent B in equation (1), with a maximum estimated error of ±0.04 eV per molecule (at 12.6 μm wavelength). Curve *l*–*l*′ shows laboratory values from ref. 3 scaled to 290 K and density 4.8 g m^{-3}, and *L*–*L*′ is the corresponding laboratory temperature dependence. Curve *m*–*m*′ shows a monomer model spectrum from the data in Table 1, for 290 K and density 4.8 g m^{-3}. The values given by *P* and *Q* are taken from ref. 1, and apply to the temperature range 258 to 299 K.

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Table 1 Sources of data used to compute theoretical absorption spectra of monomeric water vapour and carbon dioxide in the range 8–14 μm (1,250–710 cm^{-1}) and estimates for scattering

Molecular absorption model	
Line shape	Gross[12] as formulated by Zhevakin and Naumov[13] and with corrections noted by Emery[14].
Centre frequencies Line intensities	McClatchey *et al.*[15].
Air broadened line widths	Benedict and Kaplan[16].
Criteria for inclusion in the computation	
I 601 H_2O lines in the interval 710–1,250 cm^{-1} 849 CO_2 lines in the interval 710–1,250 cm^{-1}	Lines which, at their peak, contribute 0.004 dB km^{-1} or more absorption for 4.6 torr water vapour pressure (or 330 p.p.m. CO_2) when broadened by 1 atm. of air at 273 K.
II 16 H_2O lines in the interval 500–710 cm^{-1} 377 H_2O lines in the interval 1,250–1,800 cm^{-1} 358 CO_2 lines in the interval 0–710 cm^{-1}	Lines which contribute 0.0004 dB km^{-1} or more absorption at the edges of the 710–1,250 cm^{-1} band in the above condition. In this category there are no H_2O lines above 1,773 cm^{-1} and no CO_2 lines above 1,250 cm^{-1}. No H_2O lines below 500 cm^{-1} were included.
Scattering model	When the visibility is >40 km the 0.63 μm attenuation is <0.4 dB km^{-1}. For all infrared wavelengths we have assumed a scattering component of one-tenth of the value at 0.63 μm wavelength. The average expected spectral dependence for the scattering component shows a monotonic decrease from its value at 8 μm to half the value at 14 μm wavelength.

which were specified by their mean values of 281 K and 290 K respectively.

Figure 1 shows that the measured anomalous absorption (curves *a–a′* and *b–b′*) is on average several times greater than both the monomer model absorption (curve *m–m′*) and the excess absorption which was measured in the laboratory by Burch[3] (curve *l–l′*). The spectral shapes *a–a′* and *b–b′* are also different from both the model and laboratory results. In the absence of a theoretical prediction of the strength and shape of the dimer spectrum at these wavelengths, such results by themselves give little insight to molecular models. The variation of absorption with temperature, however, is predicted by the dimer model to have a temperature exponent *B* equal to the intermolecular binding energy and this can be determined independently. Values from 0.12 to 0.16 eV per molecule are given from two different sources. One is derived from the temperature dependence of the second virial coefficient[4] and the other comes from *ab initio* calculations of the total electronic binding energy and the change in vibrational zero point energy on dimerisation[5,6]. Figure 1 shows that while the temperature exponent of absorption measured in the laboratory by Burch (curve *L–L′*) can reasonably be identified with the equilibrium dimer binding energy, the values which we measured in the real atmosphere (curves *A–A′* and *B–B′*) cannot. These are on average significantly higher, with the greatest divergence between laboratory and field results appearing at the lower mean temperature. We conclude from this that an explanation of anomalous absorption being due to water dimers for all atmospheric conditions is untenable.

In relating our results to those of other workers, Coffey[1] provides a useful survey of available data. It also includes new observations which give an even higher temperature dependence as shown by the points *P* and *Q* in Fig. 1, and it is surprising therefore that Coffey concludes that they support the equilibrium dimer hypothesis. The discrepancies between temperatures determined radiometrically from satellite observations and those made '*in situ*' also suggest that a dimer model is inadequate[7]. We note that some of the discrepancies reported are greatest at low temperatures[8] which is in line with the present results.

No alternative molecular model is yet available to explain anomalous absorption. As a pointer to possible mechanisms we note that there are other results for water vapour which show anomalously high temperature exponents at low temperatures. These are from measurements of the speed of sound[9] and of absorption[10] in the region of 7 cm^{-1}. However, as such steep temperature dependence is not predicted from equilibrium properties, we propose that the phenomena must be attributed to non-equilibrium molecular complexes of water. There is some support for this notion in the fluctuations of absorption which appeared in our measurements. These were about three times greater than could be attributed to detector noise and were too large to be caused by refractive angular scintillation. The results suggest that the dissipative absorption may itself be a time-dependent quantity whose relaxation is responsible for the fluctuations. Such variability has been reported elsewhere[11].

Our results show that new molecular phenomena may have to be considered in the search for a better understanding of 10-μm region absorption and the importance of satellite radiometry alone points to the pressing need for this. If, as we suggest, non-equilibrium phenomena do play a part, this will complicate modelling but may bring some benefit, as it suggests that a systematic study of absorption fluctuations could lead to an improved understanding of the underlying physics.

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Effects on prolific petroleum source rocks and major coal deposits caused by sea-level changes

THE nature and abundance of planktonic organic matter—the main source of crude oil—and moreover its preservation in sediments are affected by sea-level changes. Deposition of paralic coals might be also influenced. It is shown here that these

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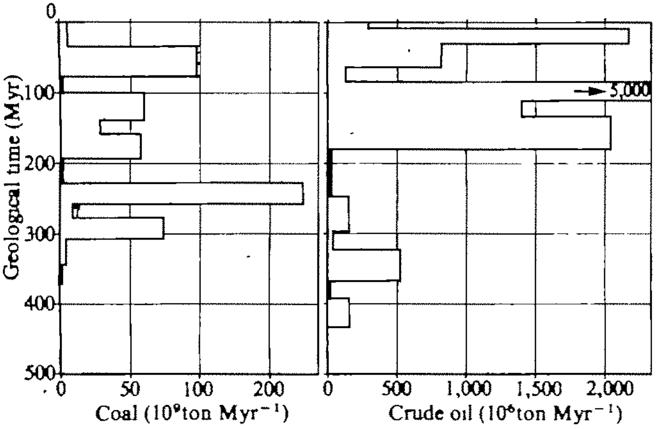


Fig. 1 Distribution of coal and petroleum as a function of geological time. The surface of the block areas is proportional to the total resources (in place, not necessarily recoverable) in geological series of each period. Data on coals, from Strakhov[1], are related to world reserves. Data on crude oil, from Bois *et al.*[2] are related to 148 petroleum zones, amounting to ~85% of the world resources. The age plotted for crude oil is the age of the source rock.

considerations would explain large accumulations of coal or petroleum source rocks over relatively short periods of time.

Available evidence indicates that coal and petroleum are unevenly distributed among geological periods. Figure 1 shows the time distribution of coal (world reserves[1]) and petroleum source rocks. The latter is plotted in terms of total—not necessarily recoverable—oil stored in reservoirs derived from the source rock. The plot includes 148 petroleum zones (ref. 2 and C. Bois, personal communication) of the world amounting to ~400×10^9 tons of oil in reservoirs. Prolific petroleum source rocks, deposited over part of Jurassic and Cretaceous (from 180 to 85 Myr) are responsible for 70% of the crude oil in these 148 petroleum zones; however, this interval amounts to 17% only of the time elapsed since the Precambrian. Considerable accumulations of coal occurred during Middle and late Carboniferous, Middle and Upper Permian, Lower and Middle Jurassic, Lower Cretaceous, and uppermost Cretaceous to Eocene. These intervals amount to 30% only of the time elapsed since the Precambrian, but they integrate ~95% of the world reserves of coal.

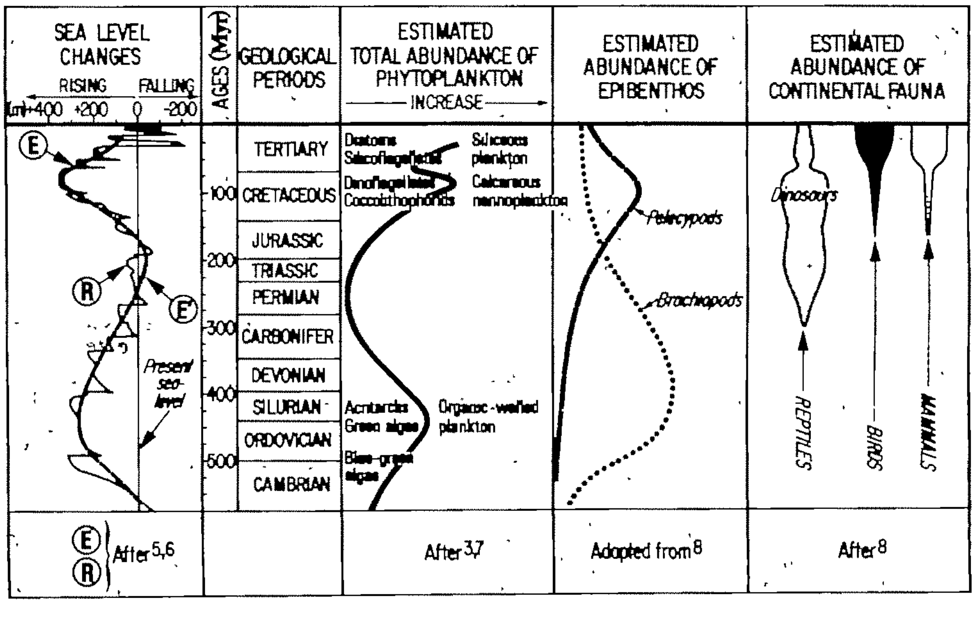
Organic geochemical studies have shown that source rocks able to generate large quantities of oil have specific characteristics. They contain an abundant organic matter of planktonic and/or bacterial origin, preserved and deposited in a reducing environment[3]. The resulting kerogen belongs to types I or II defined by Tissot *et al.*[4] on the basis of elemental analysis and infrared (IR) spectroscopy. However, the cause for accumulating large quantities of this material over wide areas during certain periods of time was not clear.

Recent developments in marine geology and geophysics made possible a comparison of these events with global sea-level changes. Pitman[5] showed that absolute rise or fall of sea-level are caused by the volume changes of the mid-oceanic ridge system. These changes are mainly due to change in spreading rate and to creation or destruction of a ridge system. The sea level (curve E, Fig. 2) was ~+350 m above present in the late Cretaceous (85 Myr) and fell to +60 m in Middle Miocene (15 Myr). Since that time glacial fluctuations may have dominated sea-level changes. Vail[6] studied the relative changes of sea level, that is, its rise or fall with respect to land surface, from the onlap of coastal deposits in seismic records of maritime sequences (curve R, Fig. 2). Both curves E and R show a good agreement, with respect to general trend and average value. Thus, it is possible to extend curve E from Cretaceous to Palaeozoic by E′ (averaging the general trend of R): two major cycles[6] of sea-level rise and fall occurred from Cambrian to Triassic (570–200 Myr) and from Jurassic to present (200–0 Myr).

Productivity and composition of marine phytoplankton—the main primary producer of marine organic matter—seems greatly influenced by these global cycles. An estimation of its total abundance shows two distinct maxima (Fig. 2) corresponding to the highest sea level, the largest extension of epicontinental seas, which are more productive than deep oceans[3]. Furthermore, the first cycle was governed by organic-walled plankton (acritarchs, green and blue-green algae), whereas the second cycle was dominated by calcareous nannoplankton (coccolithophorids, dinoflagellates) and later siliceous plankton (silicoflagellates, diatoms[3]). Dominant epibenthic faunas[8] were also replaced. On land, the disappearance of lowland plains in late Cretaceous time (sea level 350 m above present) may also be responsible for some dramatic changes of fauna.

Preservation of organic matter is a crucial point for deposition of rich source rocks. The main factors of destruction of this material are feeding heterotrophic animals and biochemical degradation by aerobic bacteria: thus the absence of oxygen in waters and in freshly deposited sediments is the main condition for preservation[9]. Shallow epicontinental seas transgressive over continental depressions often fulfill this requirement. The sea is surrounded by emergent lands, providing mineral nutrients and favouring algal blooms. In turn, the blooms resulted in an abundant bacterial consumption of oxygen and provided temporary anoxic conditions in the landlocked sea, where waters were not sufficiently renewed[10]. In deeper waters, anoxic

Fig. 2 Global cycles of absolute (E) and relative (R) sea-level changes, according to Pitman[5] and Vail[6], and their influence on marine and terrestrial populations. Estimated abundance of phytoplankton[7] shows two maxima corresponding to the highest sea-level positions. Its dominant composition[3] changes from one cycle to the other, and also that of epibenthos[8]. Terrestrial faunas[8] are also drastically changed after the highest sea-level of late Cretaceous.



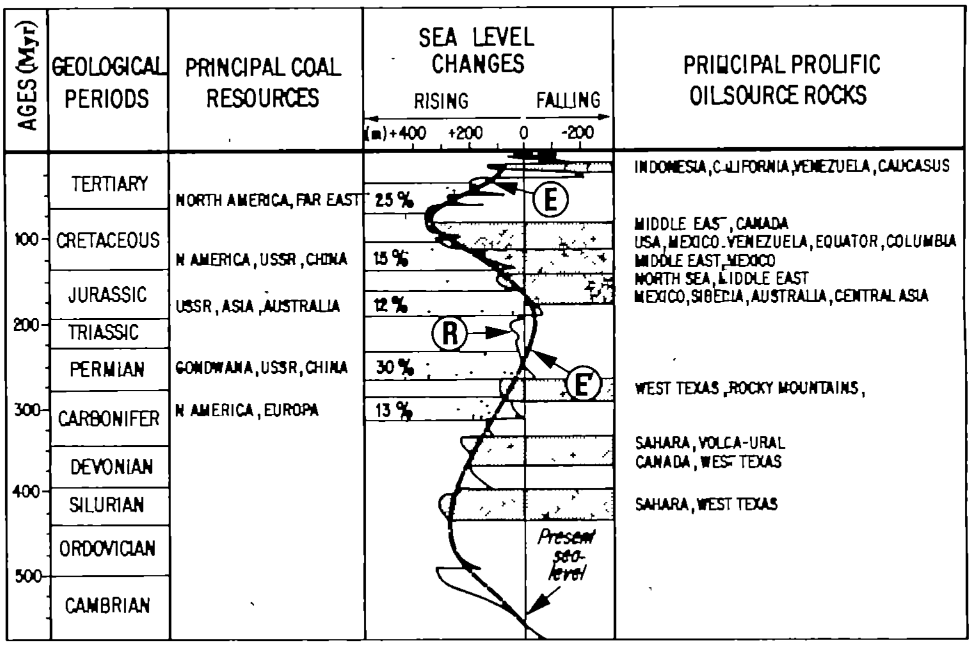


Fig. 3 Principal periods of deposition of prolific oil source rocks and major coal deposits are compared with worldwide transgressions and regressions. The periods considered for petroleum source rocks are responsible for more than 10^8 tons of oil Myr^{-1} during the first cycle (570–200 Myr) and more than 10^9 tons Myr^{-1} during the second cycle (200–0 Myr).

basins, such as the Black Sea, or areas associated with upwelling may provide comparable conditions.

Worldwide transgressions over the continental platform have been observed. Transgressions and regressions result from the balance of the rate of sea level rise or fall and the rate of subsidence minus sedimentation rate. Thus worldwide transgressions may be caused by changes of the rate of sea level rise or fall[5]. The second-order cycles of curve R (Fig. 2) illustrate these events[6]: a transgression appears as a rise of R with a positive departure compared to E–E′; a regression appears as a fall of R with a negative departure. The comparison of these cycles with the major occurrences of prolific oil source rocks (Fig. 3) indicates that they were mainly deposited during periods of global marine transgressions. For instance, the Silurian transgression deposited the rich source rocks of the Sahara over the glacial topography inherited from the Ordovician continent. In Western Europe, the successive Jurassic transgressions over the Triassic continent deposited the source rocks of the North Sea and the Toarcian and Kimmeridgian oil shales (France, Germany, UK).

However, the potential for oil generation seems to be lower by about 1 : 10 in the older cycle, as compared to the younger one. This is probably due to a greater thermal maturation—generating gas—and to tectonic events (faults, folding and erosion) which have destroyed a large part of the oil originally formed.

Most important coal occurrences were formed by large accumulations of terrestrial higher plants in coastal or paralic basins. A long-lasting subsidence is necessary to provide thick series of shallow deposits and a moderate relief of the hinterland to avoid dilution by inorganic material. The most typical environment is provided by coastal lowlands with swamps which develop after regression of the sea. The comparison of the age of the major coal deposits—$> 50 \times 10^9$ tons Myr^{-1}—with the second-order cycles of curve R (Fig. 3) indicates a frequent correlation with worldwide regressive periods.

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Trace elements in the fluid phase of the Earth's mantle

It has become apparent during the past decade or so that CO_2 is a significant component of the Earth's upper mantle. In addition to the indirect evidence including CO_2 emanations from volcanoes throughout the world and the abundance of CO_2 in kimberlites and carbonatites, it is now known that CO_2 is ubiquitously present in xenoliths of mantle material brought up in alkalic basalts and kimberlites. This latter point was first made by Roedder[1] who demonstrated that the fluid in optically visible inclusions is essentially pure CO_2 under pressures as great as 5 kbar at eruptive temperatures. Such pressures provide only a minimum depth of origin, because Roedder further showed that most of the inclusions he observed are secondary, that is, they occur as arrays on healed cracks. A more recent study[2] indicated pressures as high as 10 kbar in secondary inclusions. H.W.G. and Radcliffe[3] found, using transmission electron microscopy (TEM), that submicroscopic fluid inclusions also occur within the crystals of mantle xenoliths, and that these bubbles have exsolved (precipitated) from solid solution. Thus, at least the CO_2 now present in submicroscopic fluid inclusions is indigenous to the crystals in which it is found, and probably represents the source of crack formation and filling to produce the secondary inclusions on the way to the surface[3]. The effect CO_2-bearing phases have on the distribution of trace elements in the mantle and during partial melting is considered here.

The term 'fluid inclusion' is used here as a descriptive term only; I reject the usual connotation that the fluid has been 'included' into the crystals as a growth accident in an igneous environment. For example, virtually all of the rocks in question are metamorphic and show no signs of introduction of a fluid phase from an external source. Moreover, metamorphic recrystallisation 'excludes' fluid and concentrates it onto grain boundaries, rather that 'including' it during grain growth[3].

CO_2 would appear, then, to be an important component of most, perhaps all, of the upper mantle and two questions are paramount: (1) in what phases does the CO_2 reside in the upper mantle?; and (2) what effect do these CO_2-bearing phases have on the partitioning of trace elements within the mantle and between melt and residuum during partial melting? The former is easier to answer. There now seems to be too much CO_2 (at

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least in the uppermost mantle) for it simply to be dissolved in the silicates as originally assumed by H.W.G.[4]; moreover, recent experimental work indicates that in the mantle beneath undisturbed continents, the CO_2-bearing phase is probably a carbonate[5-7] and beneath oceans the stable phase may be a carbonate[5,6] or supercritical fluid[7]. All the data strongly suggest, however, that because of the steeper geothermal gradient in the vicinity of an oceanic ridge, the stable phase in the lithosphere under ridges will be fluid CO_2. The second question is more difficult to answer, but is fundamentally more important because the concentrations of trace elements in melts derived from the mantle are routinely used to infer the present composition of the upper mantle and its geochemical evolution. These inferences are always based on calculated or experimentally determined element partitioning between basaltic melt and mantle silicates, especially clinopyroxene and garnet. If significant quantities of these elements reside in another phase—a CO_2-bearing phase—and if portions of this phase are not incorporated into the melt, then current interpretations of trace element data could be in error. The ubiquitous occurrence of CO_2 inclusions in refractory xenoliths such as harzburgite and dunite indicates that melt extraction does not scavenge all of the CO_2-bearing phase.

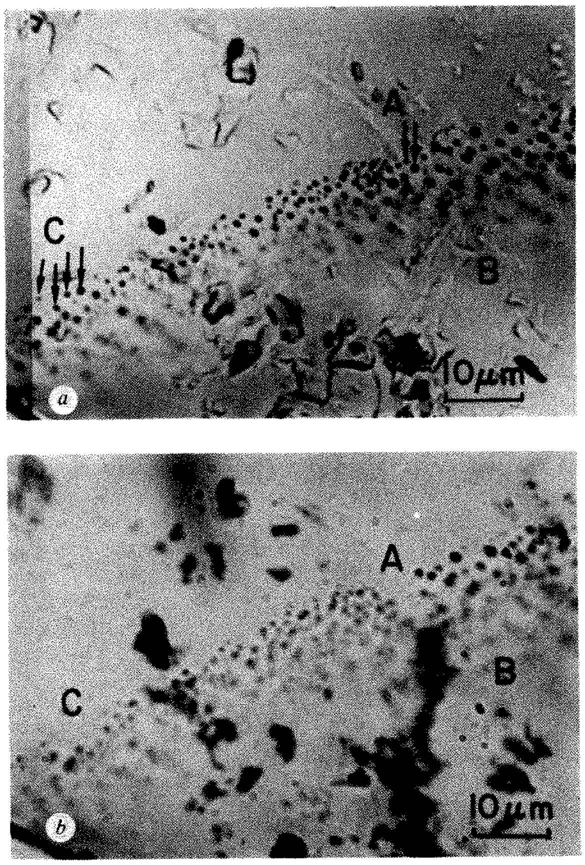

Fig. 1 Photomicrographs showing region of olivine specimen H-1 before (*a*) and after (*b*) ion probing. A healed crack decorated with 2-phase CO_2 bubbles intersects the top of the thin section along line AC. Arrows in (*a*) point to bubbles which were removed by sputtering and are absent in (*b*). Irregular black and grey areas in (*b*) are surface pits from which the gold film could not be removed after probing.

As a first step to determination of the trace element problem, an ion probe study has been inaugurated to attempt detection of trace elements in CO_2 inclusions[8]. The procedure chosen was to 'drill' down through host crystal and break bubbles. To simplify the initial search, elements known to be major components of carbonatites and to have no good crystallographic home in mantle phases (olivine, orthopyroxene, clinopyroxene, garnet, spinel) were chosen as good candidates. In addition, mass numbers known to give interfering peaks produced by the matrix elements and polyatomic reaction products of matrix elements with the ^{16}O beam were omitted. For example, ^{12}C, ^{28}CO, $^{44}CO_2$, ^{40}Ca, ^{40}Ar were not attempted due to interference with $^{24}Mg^{2+}$, ^{28}Si, ^{44}SiO and ^{40}MgO respectively. Five or six different mass numbers were searched in each run, including trace elements and elements to serve as internal monitors of the matrix, such as ^{24}Mg. The results were positive for masses 39(K) and 47(Ti), suggestive for mass 140(Ce) and inconclusive for masses 1(H), 85(Rb), 86(Sr), 87(Sr), 138(Ba), 142(Ce) and 208(Pb).

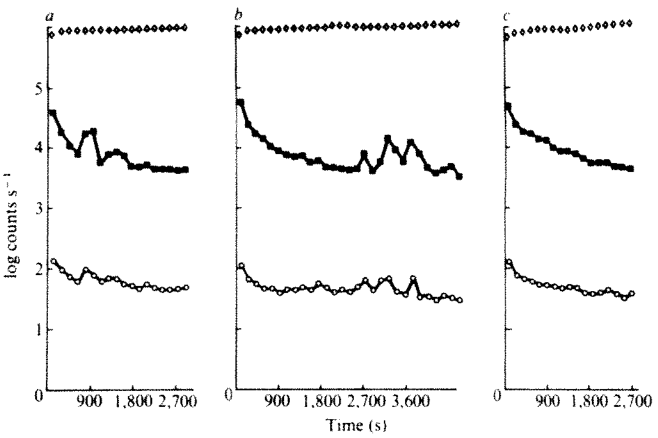

Fig. 2 Depth profiles for masses 24, 39 and 47 obtained from areas A, (*a*) C, (*b*) and B (*c*) of Fig. 1. Area B is a control profile obtained where no (optically visible) bubbles were present. ■, ^{39}K; ○, ^{47}Ti; ◇, ^{24}Mg.

A portion of a bubble array on a healed crack in olivine of dunite specimen no. H-1 from the 1801 Kaupulehu lava flow of Hualalai volcano, Hawaii, is shown in Fig. 1*a* before ion probing and in Fig. 1*b* after probing. At site C, four inclusions were removed during bombardment of the surface for 80 min with a 15 nA ^{16}O beam of elliptical cross-section (~8 µm × 15 µm). Mass numbers were examined sequentially with each mass counted for 5 s, except for ^{24}Mg which was counted for 3 s. The concentration profile produced during the erosion at site C is given in Fig. 2*b*. The four distinct peaks in masses 39 and 47 are presumed to correlate with the four bubbles removed by sputtering; no corresponding peaks (or valleys) in ^{24}Mg are observed.

Another profile measured at site A of the same inclusion array broke two bubbles (Fig. 1) and shows two peaks (Fig. 2*a*), whereas a control profile measured at site B in the same olivine crystal in an area of no fluid inclusions shows no peaks (Fig. 2*c*). Figure 3 shows similar results for an olivine crystal of lherzolite specimen No. SAL-3 from Salt Lake Crater, Oahu, Hawaii, and Fig. 4 shows the results of a long profile of an orthopyroxene crystal of this same specimen. The apparent noise in the mass 39 data of the latter profile is produced by breaking numerous small inclusions (≤ 1 µm diameter) and three larger ones (~5 µm diameter). Of particular interest in the mass 39 profile of Fig. 4 is the large, double peak between 6,200 and 7,400 s, and the secondary one between 7,800 and 8,500 s. These probably represent penetration of the three larger bubbles. These peaks correspond exactly to the highest values of the mass 140 profile which also shows a large, divided peak and a narrower one. Although the maximum count-rate is 4.5 c s⁻¹ for mass 140, this represents 2.9 standard deviations above the mean of all data points after surface contamination had been removed (2,700 s). Similarly, the two subsidiary peaks represent 1.8 standard deviations above the mean. Thus, the data can reasonably be considered significant on their own, and correspondence with the largest peaks in mass 39 increases the likelihood that these inclusions contain detectable amounts of cerium.

Quantitative interpretation of these data depend on the ionisation and collection efficiency of the ions involved, which, in turn, critically depend on whether the elements in question are in the fluid itself or are present as a solid film on the surface of the bubbles. For potassium, for instance, the fraction of atoms detected from a fluid is of the order of 10^{-3}, but from a solid substrate is ~0.6 (T. Whatley, personal communication). Two lines of evidence strongly suggest that the trace elements detected here are in a surface film: (1) If the efficiency of detection is 10^{-3}, the K content of the bubbles in Fig. 2 is >10%, an apparently unreasonably high value. On the other hand, if the detection efficiency is 0.6, the K content is about 1,000 p.p.m. of the fluid contents; (2) the peaks last too long. The best example of this phenomenon is the double peak in Fig. 4, which lasts for 20 min. During this time the beam will have eroded about 0.5 μm deeper into the pyroxene crystal and opened an orifice as large as 3 μm across on the 5 μm bubble(s). We can expect that the fluid, under very high pressure, would escape in the first few seconds of this time, hence the mass 39 and 140 elements cannot be in the fluid phase itself. It would seem most reasonable to conclude, therefore, that the trace elements detected in this study were originally in the fluid at high pressure and high temperature, but are now located in a film on the bubble walls; after penetration of the bubble, the ion beam 'washes out' the thin film.

Masses 39 and 47 which have been definitely detected here, are not uniquely interpreted as K and Ti; they could be ^{39}NaO and ^{47}PO. Na is unlikely to be a major contributor to the peaks reported here because the vast majority of Na is always registered at mass 23. A significant contribution of Na at mass 39 would therefore imply a very large Na content to the inclusions, a composition inconsistent with the conventional fluid inclusion studies[1,2]. ^{31}P, however, is commonly detected as ^{47}PO with an ^{16}O beam (T. Whatley, personal communication) and hence may be a significant, even dominant contributor to the peaks at mass 47. In this regard it is important to distinguish the results of this study and the analysis of glassy rims sometimes found on fluid inclusions in these rocks. Although K, Na, Ti and P have been detected in such rims[2], the surface film detected here seems to be a different phenomenon. Most inclusion arrays in peridotite xenoliths contain only fluid. This has been determined by optical observation of the brownian motion of the inner vapour bubble when the inclusions are sufficiently large (≫2 μm diameter), and by TEM when they are not. In every array investigated, when all of the large inclusions show an inner bubble in motion and no glass rim, the TEM confirms the absence of a glass rim on the smaller inclusions. Some submicroscopic inclusions, however, do suggest a very thin (<100 Å) surface film, which I have so far been unable to convince myself was definitely present. Furthermore, the three larger inclusions originally present in specimen SAL 3-14 (Fig. 4) displayed moving vapour bubbles and lacked a glassy rim. The volume fraction of fluid is thus very much larger in the inclusions described here than in those with glassy rims figured by Roedder[1] and Murck *et al.*[2], and it would seem difficult to interpret my results as evidence of an immiscible silicate liquid incorporated along with the CO_2. It seems more likely that these elements were dissolved in the fluid at high pressure.

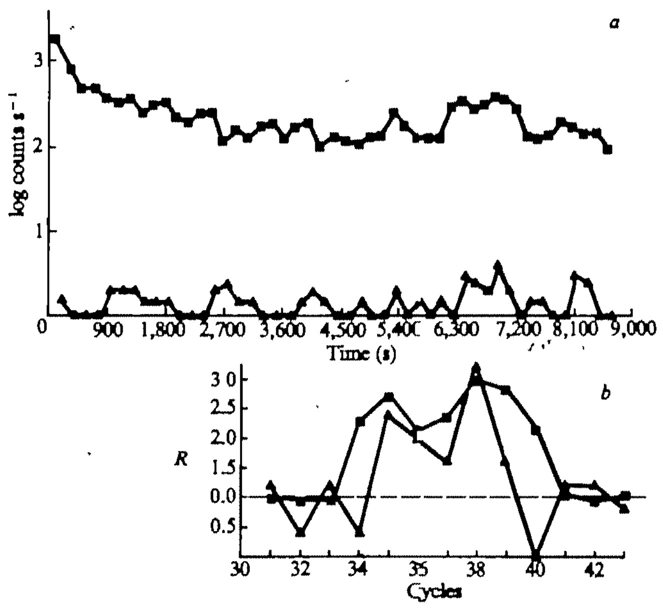

Fig. 4 Depth profile for masses 39 and 140 for area 14 of specimen SAL-3. Large doublet, peak between 6,200 and 7,400 s (cycles 33–43) is replotted in (*b*) in terms of the ratio (*R*) of counts s^{-1} divided by the average counts s^{-1} for cycles 33–43. ■, ^{39}K; ▲, ^{140}Ce

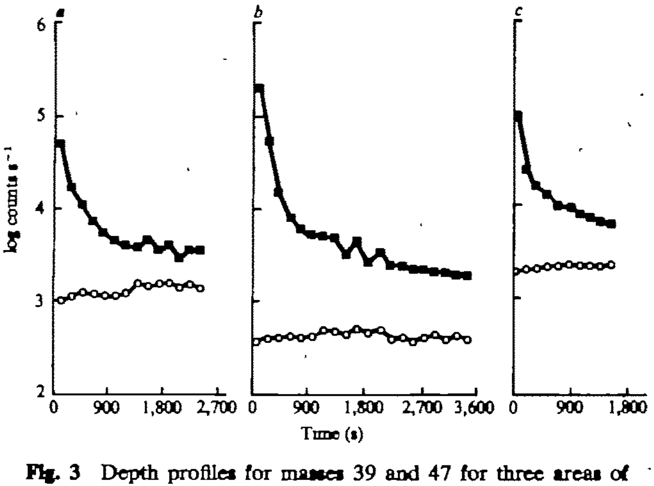

Fig. 3 Depth profiles for masses 39 and 47 for three areas of specimen SAL-3: *a*, SAL-3-14A; *b*, SAL-3-26A; *c*, SAL-3-25A. Profile 25A is a control obtained where no (optically visible) bubbles were present. ■, ^{39}K; ○, ^{47}Ti.

The results of this study demonstrate that the CO_2-bearing phase in the mantle contains significant quantities of cations other than carbon. They do not, however, help much in determination of whether the CO_2 was already a fluid or was in a carbonate when it began its trip to the surface (because the inclusions analysed are secondary and could conceivably represent the product of carbonate breakdown). However, other workers have shown that the CO_2 bubbles in xenoliths also contain significant quantities of noble gases[9–11]. Of major interest is the detection of excess ^{129}Xe (ref. 11) with its implication that the Earth formed within 76 Myr of nucleosynthesis[12] and has not been totally outgassed since that time[11,12]. Another point of major importance is the large quantity of ^{40}Ar, giving anomalously old K/Ar ages. These latter observations suggest that the CO_2-bearing phase has acted as a sink for rare gases—a property which is more likely to characterise a fluid than a carbonate.

Finally, the detection of alkalis and a suggestion of light rare earths suggest caution in interpretation of mantle compositions from basalt chemistry using silicate-liquid partition coefficients.

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Speciation of dissolved iodine in estuarine waters

IODINE in seawater is present as iodate (IO$_3^-$) and as iodide (I$^-$)[1-3]. Although there is some uncertainty in the true redox potentials of natural waters[4], the thermodynamically stable species of iodine in seawater, and in river waters which are well oxygenated, is iodate[5-7]. We have studied an estuary to determine the distribution of iodine species as a function of salinity, and examined the results for evidence of interconversion of species in the estuary. We report here that iodide is the dominant form in river water and is oxidised to the thermodynamically stable iodate in the sea, not in the estuary.

Our study area was the estuary of the Yarra River, which drains part of southern Victoria, Australia, and includes the city of Melbourne with 1,200,000 inhabitants in its catchment. The estuary is 22 km long and highly stratified[8], but not oxygen deficient. The river discharges into Port Phillip Bay, a large embayment with only a small outlet to Bass Strait. The survey was made in late winter at a time of high river flow (22 August 1978). Water samples were filtered through prewashed Gelman 0.45 μm membrane filters, stored in glass bottles at 4 °C in the dark, and analysed within a month of collection. Previous storage experiments showed that no measurable changes in iodate or iodide occurred in water stored in these conditions.

Iodate in seawater, and in estuarine waters of salinity >2‰ was determined directly by differential pulse polarography (DPP)[9,10]. A 20-ml aliquot of the water sample was pipetted into a polarographic cell, EDTA solution was added to suppress the zinc wave, and the solution deoxygenated by bubbling argon. A Metrohm E500 series Polarecord with a dropping mercury electrode, platinum wire counter electrode, and Ag/AgCl/M KCl reference electrode, was used. Concentration of iodate was determined by the method of standard additions. Iodide was determined in a separate aliquot of the sample by oxidation of iodide to iodate by addition of chlorine water[11], evaluation of the new level of iodate by the polarographic method, and calculation of iodide by difference. We found ultraviolet (UV) irradiation[10] unsuitable for iodide determination, as it produced peroxide that interfered and also gave iodate from 'organo'-iodine compounds in estuarine waters. No interfering species were produced by chlorine water in the experimental conditions used. In river water, the iodate-plus-iodide concentration could not be found by simple addition of chlorine water, as the large amount of dissolved organic matter interfered with the determination. A modified procedure was developed, in which a 50-ml aliquot of river water was treated with sodium hydroxide and chlorine water, then evaporated to dryness under an infrared lamp. The residue was redissolved in 10 ml of doubly distilled, deionised water, this solution treated with chlorine water, and sodium formate solution added. The resulting solution was filtered to remove coagulated organic matter and made up to 25 ml with water. Iodate-plus-iodide was then determined on a 20-ml aliquot after addition of EDTA. Analysis of water of salinity 5‰ by both procedures yielded results that were not significantly different, indicating that the more complex treatment did not convert any further iodine to iodate.

Replicate analyses of a bulk estuarine sample of salinity 19.4‰, collected at an earlier date, gave 7.9 μg l^{-1} I as iodate with a coefficient of variation of 6.7%; 53.4 μg l^{-1} I as iodate-plus-iodide with a coefficient of variation of 3.2%; and by difference, 45.5 μg l^{-1} I as iodide with a coefficient of variation of 9.9%. The limit of detection of iodate is largely dependent on establishment of the baseline of the polarograms, but taking two standard deviations at the 7.9 μg l^{-1} level it is about 1.0 μg l^{-1} I. Salinity was measured using an AutoLab 602 salinometer calibrated using IAPSO standard seawater.

Results from the Yarra River estuary (Fig. 1) indicate that both iodide and iodate show conservative behaviour. The river water contained 23 μg l^{-1} I as iodide, and <1.0 μg l^{-1} as iodate. The seawater contained 28 μg l^{-1} I as iodate, and 36 μg l^{-1} as iodide, as the average of five samples with a mean salinity of 34.0‰. 'Iodine', as iodide-plus-iodate, determined after oxidation with chlorine water, was 23 μg l^{-1} in the Yarra river water which is within the range of 2.2 to 42.4 μg l^{-1} reported for iodine in other rivers of the world[12]. The dominant form of iodine in rocks and soils is iodide. This is likely to be the case in the Yarra drainage basin, and apparently no conversion to iodate occurs in the transit from source mineral to the river. 'Iodine' in seawater of 34‰ salinity from Port Phillip Bay at 64 μg l^{-1} I is higher than seawater values reported by Truesdale[13], but comparable with the average values of Barkley and Thompson[3] and Wong[14].

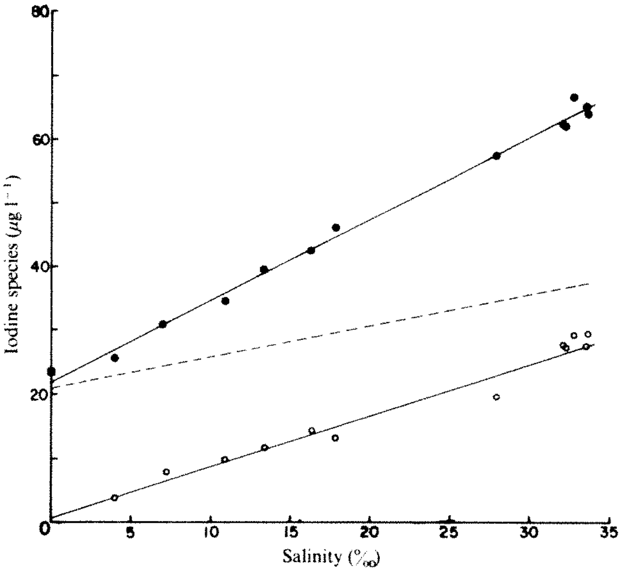

Fig. 1 Relationship between salinity and concentration of iodate (○) and iodate-plus-iodide (●). Lines of best fit are shown, and the dashed line represents the iodide concentration determined by difference. The concentration of iodate-plus-iodide in river water (■) was determined by a procedure different from that used for estuarine waters.

The present study indicates that there is no measurable conversion of the iodide of river water to iodate within the Yarra River estuary, and that the iodate in the estuary comes from the seawater. Consequently, it seems that oxidation of iodide to the thermodynamically stable iodate occurs in the seawater outside the estuaries. Two mechanisms, which are known to effect oxidation of iodide to iodate occur through UV irradiation, and by biological activity of algae. We have used UV irradiation for conversion of iodide to iodate as an analytical technique, and found that quantitative conversion of iodide to iodate required ~3 h exposure to a 1,200 W mercury lamp at a distance of 6 cm. Sunlight would be expected to affect only the surface water, and then at a very slow rate. The effects of algae on iodide and iodate in seawater have been reported. Sugawara and Terada[15] found that a diatom culture in seawater containing 16 μg l^{-1} iodide converted 3 μg l^{-1} iodide to iodate in four weeks. Truesdale[13] found that some *Chlorophycae*, cultured in seawater containing 50 μg l^{-1} interconverted not more than 5 μg l^{-1} in an unspecified time.

Although we did not observe any apparent reaction of iodide or iodate in the estuary, we did detect the presence of a third iodine form. When we exposed waters of intermediate salinity to UV irradiation for 4 h before addition of chlorine water, we observed an increase in the iodate detectable by DPP compared with that found after oxidation using chlorine water alone. Our experiments with 2-iodo-benzoic acid in the conditions used in analysis of estuarine waters showed that iodide in this compound was not converted to iodate by treatment with chlorine water, whereas UV irradiation followed by treatment with chlorine

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water gave quantitative recovery of the organo–iodine as iodate determined by DPP. The estuarine water of salinity 13.4‰ contained 27.9 μg l^{-1} I as iodide, 11.6 μg l^{-1} as iodate, and a further 7.0 μg l^{-1} released after UV irradiation and treatment with chlorine water. The increase is probably the result of decomposition of organo–iodine compounds by the UV irradiation, and 15% of the total iodine was present in this estuarine water as 'organo'–iodine. The seawater of 34‰ did not yield additional iodate when UV irradiated before chlorination. Organo–iodine has been reported in seawater by Truesdale[16] but there are no previous reports of iodine speciation in estuarine or river waters.

The results of Truesdale[13] show a relatively constant 'total' iodine in seawaters, with a decrease in iodide and increase in iodate progressing from Menai Strait, through the Irish Sea, to North Atlantic Ocean waters. He suggests that iodate-reducing substances introduced to the sea by terrestrial run-off might explain these observations.

On the basis of our evidence, variations of iodide to iodate ratio might also be largely explained as the result of oxidation of iodide delivered by rivers to the oceans. The production of iodate seems to be thermodynamically favoured, but limited by the slow rate of conversion, with a reported half life of tens of years[17]. This is too slow for it to be observed in the Yarra River estuary, where residence time is only days. Hence conversion of iodide to iodate must occur in the ocean, possibly under the influence of enzyme systems.

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Effect of glacial ice melting on the Antarctic Surface Water

MOST glacial ice melting occurs below the sea surface and in regions where ice shelves and icebergs are concentrated, as along the Antarctic coastline. We show here that this melting can significantly affect the Antarctic Surface Water (AASW) chemistry and biology only for glacial ice constituents are greatly depleted (for example, salt, oxygen-18) or enhanced (for example, ammonia?) relative to the ocean. The surface water nutrient (nitrate, phosphate, silicate) budget is controlled primarily by the upwelling of deep water. Melting beneath the Ross Ice Shelf results in the northwards lateral movement of near-freezing water to the continental shelf break. Basal melting of icebergs and smaller ice shelves may contribute to the temperature minimum at the base of the AASW.

The volume of meltwater produced annually from melting Antarctic glacial ice will be three orders of magnitude less than the AASW reservoir, which extends from 50 to 200 m depths over the 20 × 10^6 km^2 area south of the Polar Front (Antarctic convergence). For an Antarctic ice cap in an equilibrium state, the average annual volume of glacial ice lost must equal the 0.15 m yr^{-1} net accumulation over the 14 × 10^6 km^2 area of Antarctica[1]. We assume relatively little ablation[2]. Net deep melting of 0.30 m yr^{-1} (refs 3, 4) beneath the large ice shelves, which comprise less than 11% of the ice cap[4,5], would account for no more than a quarter of the 2.1 × 10^3 km^3 wastage. The remaining ice will melt at shallower levels, within or near the base of AASW from icebergs, ice streams, small ice shelves and from the northern edges of the major ice shelves.

Parker *et al.*[6] have suggested that nitrogen-laden ice originating on the Antarctic continent provides, on melting, a major contribution (7% yr^{-1}) to the nitrogen budget of the 50 m epipelagic zone of the Antarctic oceans. Since then Biggs has shown[7] that Parker *et al.* used incorrect nitrate (NO_3–N) values, and he has recomputed the nitrate input from continental ice as 0.02% of that in the AASW, assuming a surface mean of 12 μg-atoms l^{-1}. However, there are significant differences between the nitrate observations reported by various expeditions in the Antarctic oceans (Fig. 4 in ref. 8). Our AASW nitrate measurements[8,9] and those of the Geosecs expeditions[10] range from 15–30 μg-atoms l^{-1}, with a mean around 22 μg-atoms l^{-1}. With this higher nitrate mean, and a reservoir thickness closer to 100 m than to 50 m, the net nitrate input from glacial ice would be 0.005% of that already in the AASW.

The Parker *et al.*[6] nitrate mean of 19.57 μg l^{-1} (1.4 μg-atoms l^{-1}) is an order of magnitude less than the AASW nitrate concentration, so iceberg melting would cause a slight nitrate decrease locally in the AASW rather than an enrichment as claimed. Subsequently, Parker *et al.*[11] reported nitrate values as high as 37 μg-atoms l^{-1} in additional ice samples, but they do not indicate whether their nitrate mean has increased or decreased from 1.4 μg-atoms l^{-1}. We have made nitrogen measurements on glacial ice recovered from the sea near a green iceberg[12] and on an ice core sample from 66 m below the surface of the Ross Ice Shelf at 82°22.5′S, 168°37.5′W. The nitrate assay was 3.9 μg-atoms l^{-1} in the iceberg, and 1.1 μg-atoms l^{-1} in the ice core, consistent with the earlier Parker *et al.* data.

Nitrate, phosphate and silicate are the nutrients commonly measured in seawater. These nutrients are present in abundance in the AASW south of the Polar Front and are not generally believed to limit phytoplankton growth. If other compounds such as ammonia (NH_4–N) are limiting to the rate of primary production[7], then melting glacial ice may be an important factor, locally. Our ammonia value for the ice shelf core sample (2.4 μg-atoms l^{-1}) is similar to the Parker *et al.* mean (1 μg-atoms l^{-1}). Both measurements are an order of magnitude greater than surface water ammonia concentrations reported for the Ross Sea (< 0.1–0.3 μg-atoms l^{-1}) (ref. 7). The ammonia levels of aliquots drawn from the atypical green iceberg sample ranged from 25 to 33 μg-atoms l^{-1}, slightly above the range for South Pole ice core samples[6]. The green iceberg sample also contained quartz grains and metallic particles (J. Anderson, personal communication), fibres, algal cells, and an organic nitrogen level of 241.6 μg-atoms l^{-1}. A thin section showed it to be derived from a valley glacier (A. Gow, personal communication).

Upwelled deep water is the primary source of nitrate, phosphate and silicate in the AASW. Beneath the AASW, nitrate values are uniformly above 30 μg-atoms l^{-1} (refs 9, 10). This deeper water upwells into the surface layers, replacing waters that mix and sink as Antarctic Bottom Water and Antarctic Intermediate Water. The upwelling rate, based upon temperature, salinity and oxygen-18 budgets, has been estimated to be 60 × 10^6 m^3 s^{-1} (refs 13, 14), corresponding to a nitrate input to the AASW of 8 × 10^8 tonnes yr^{-1}. This is four orders of magnitude greater than the meltwater contribution of 3 × 10^4 tonnes yr^{-1}, derived from the Parker *et al.* mean nitrate for glacial ice and the assumption of an equilibrium ice cap. Nitrate

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enrichment of 18% in the euphotic zone of a glaciated Arctic fiord was first attributed to rock flour[15] and later to upwelling[16].

Most iceberg melting occurs below the sea surface in the predominantly above-freezing environment that exists there during all seasons. Holdsworth and others have shown that the amount of downwarping of sea ice attached or frozen to icebergs in d'Iberville Fiord, NWT, Canada, may be accounted for by continuous melting of the submerged portions of the glacier ice throughout the year. Thus, there may be no sharp seasonal glacial ice melting peaks coincident with phytoplankton blooms[6].

Several associations have been reported between glacial ice and the biological community. Bottom scouring by icebergs affects bottom dwellers in several ways[17]. Links have been noted between icebergs or shelf ice and microflora[18], diatoms[19], Arctic terns[20], seals in the Arctic[17] and leopard seals in the Antarctic[21]. We have observed Antarctic petrels, snow petrels and Adelie penguins on and in the vicinity of icebergs. Glacial ice contains some 3–8% (by volume) compressed air bubbles[22] that may influence mixing processes when the ice melts. The 'sizzling' noise that accompanies the bubble pressure release "should be detectable by sonar . . . out to distances of 50–100 miles against the ambient sea background"[23]. If plankton levels are higher near icebergs, then the noise of that melting ice could well attract higher species.

There is evidence that melting glacial ice may influence vertical processes in the surface layers. Neshyba[24] has pointed out the potential importance of iceberg-induced upwelling on the oceanography of the Weddell Sea, and Foldvik and Kvinge[25] have described the possible thermohaline upwelling of near-freezing water near ice shelves. Few oceanographic observations have been made in the vicinity of Antarctic icebergs. The anomalously high subsurface salinities and temperatures recently reported near a large Antarctic iceberg[26] may be an artefact of the instrument or calibration. In the Ross Sea we have observed brash or pack ice surrounding an iceberg (see also Fig. 24 in ref. 27) but separated from it by a ring of open water. This may result from upwelling along the walls, wave and swell interaction, or differential motion between iceberg and smaller pieces of ice with shallower draft. Upwelling was considered responsible for muddy ice-free water at the sea faces of glaciers in Greenland[28]. Josberger[29] reported field measurements of melt-driven upwelling adjacent to an Arctic iceberg, and modelled both upwelling and downwelling in the laboratory. Several of his vertical temperature–depth profiles suggest additional effects at 50–70 m.

In another laboratory experiment[30] meltwater was observed to mix and spread laterally away from ice blocks inserted into salt-stratified water. Huppert and Turner noted the production of a "series of tilted convecting layers which grow out into the environment". They found that "a considerable portion of the melt water can be fed into the environment close to the level at which it is produced and very little, if any, rises to the surface".

We have measured the large scale lateral spreading of water (Fig. 1) that has been cooled by melting at the base of the Ross Ice Shelf[3]. This Ice Shelf Water[31] extends northwards to at least the continental shelf break. A similar water mass is found on the continental shelf in the Weddell Sea[32] and adjacent to the Amery Ice Shelf[33]. Ice Shelf Water temperatures are as low as the *in situ* freezing point; salinity ranges from 34.4 to 34.75‰, but does not usually form a local minimum within the vertical halocline. Ice Shelf Water with potential temperature below −2.0 °C has been observed in the Ross Sea at depths from 225 to 525 m. We have hypothesised[3] that the main source of heat for melting at the base of the Ross Ice Shelf is provided by an intermediate-depth warmer layer, derived from the Circumpolar Deep Water. Pettersson[34] seems to have modelled several of the thermohaline features we observed north of the Ross Ice Shelf.

Similar to flow away from the Ross Ice Shelf (Fig. 1) and ice in the laboratory[30], most meltwater from icebergs may spread laterally rather than upwell along the sides. Typical tabular Antarctic icebergs will have initial drafts approximating the depths below sea level of the terminal faces of the ice shelves. A study of 407 Antarctic icebergs[35] showed a mean freeboard of 40 m, corresponding to a subsurface draft of 182 m. Mean widths for icebergs observed with Landsat imagery in one Antarctic sector were 0.4 km on the edge of the pack ice and 0.7 km in the pack ice[36]. For icebergs with dimensions larger than about 0.7 km, basal area will exceed wall area. The base should then be the primary wastage surface[34] and this surface will frequently be near the lower levels of the AASW. Alternatively, if calving as a result of wave-induced melting and mechanical erosion were the primary disintegration process[37], most melting would take place nearer the sea surface. We have often observed the calving of brash and growler-sized pieces

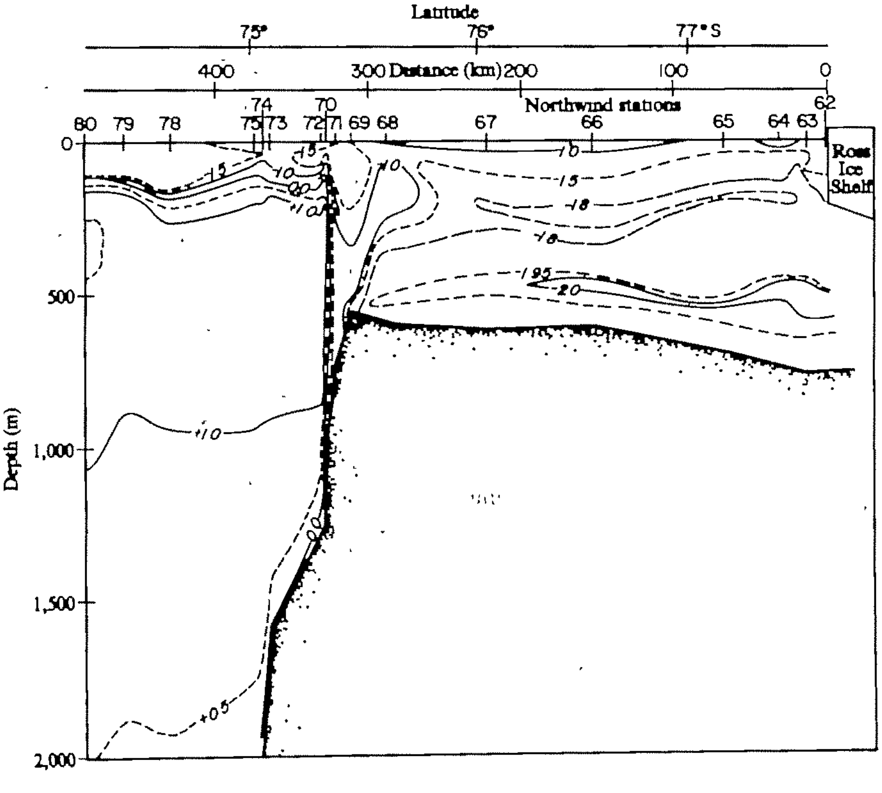

Fig. 1 Potential temperature profile from USCGC Northwind stations taken in December 1976 (ref. 46). The section extends about 500 km north-east of station 62, which was next to the Ross Ice Shelf at 178° E longitude. A layer of ice shelf water[31] ($\theta < -1.95$ °C), that results from melting beneath the ice shelf, reaches the continental shelf break. A shallower layer of ice shelf water ($\theta < -1.8$ °C) also extends north from the ice shelf at Antarctic surface water depths. An intermediate depth warmer layer ($\theta > 1.8$ °C), better developed on the east–central continental shelf, provides the major heat flux for melting of the glacial ice[3].

from the northern face of the Ross Ice Shelf, but note that much of the region populated by Antarctic icebergs and ice shelves is covered by sea ice for most of the year, which should limit wave-induced erosion.

The temperature minimum at the base of the Antarctic Surface Water is generally attributed to vertical winter mixing, with subsequent capping by a warm summer mixed layer[38]. However, the large latent heat exchange (80 cal g^{-1}) at the base of melting icebergs and subsequent lateral mixing of the meltwater could also contribute to this feature. Where the temperature minimum is shallow, as in the Weddell Gyre, the impact of iceberg melting may extend well below the AASW. The step-structure in the centre of the Weddell Gyre that Huppert and Turner attribute to "systematic injection of meltwater at intermediate depths" is best developed just below the temperature minimum in their Fig. 1*b* (ref. 30). Over the AASW region south of the Antarctic Convergence, the heat exchanged annually in melting glacial ice should be sufficient to lower by 1 °C the temperature of a layer 7.5 m thick. While the upwelling rate used above corresponds to an AASW residence time of only one year, decreasing this rate[39] would allow to a greater influence of iceberg melting on the AASW. An order of magnitude more heat will be taken up by the summer melting of pack ice than by the annual melting of glacial ice, but the melting pack ice will primarily involve only the top few metres of seawater. That meltwater will have a stabilising effect on the water column, and the associated latent heat flux will be more than offset by incoming radiation[40].

The environmental impact of melting icebergs will not be evenly distributed in space and time. Iceberg tracks will be influenced by currents, oceanic fronts, winds, pack ice, bottom topography, and by the location and productivity of drainage areas. From our observations and published accounts[35,36,42,43] of iceberg distribution and movement, we would expect the maximum impact from melting icebergs to occur in the Antarctic coastal zone. There a cold and fresh water mass, V-shaped in cross-section[44] is frequently observed over the Antarctic continental slope. It extends to depths of several hundred metres and apparently defines the deeper portion of the surface westwards coastal current[45]. Its low temperature and low salinity[44,46] probably result in part from melting glacial ice. It may be a source reservoir for both the AASW temperature minimum and the fresh component of Antarctic Bottom Water.

North of the continent, icebergs frequently appear in clusters and are likely to travel preferred routes. The gigantic Trolltunga iceberg that has been tracked by satellite for 11 years (Fig. 23 in ref. 27) may provide evidence for the association of icebergs with convergent frontal zones. Once Trolltunga entered the northern Scotia Sea it seems to have tracked the southern boundary of the Polar Front, possibly grounding as it crossed the North Scotia Ridge. Moving parallel to the bottom contours, it then executed two reversals in an east–west direction, a characteristic feature of the front in that region[47]. To the extent that icebergs are associated with frontal zones[48], the meltwater may contribute to lower surface salinities sometimes observed there (Fig. 4*b* in ref. 49).

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Isolation and characterisation of a bipotential haematopoietic cell line

VARIOUS cell lines have been used to study the regulation of proliferation and differentiation, and events occurring during mutagenesis and carcinogenesis. These include 'normal' cell lines which, however, frequently show chromosome changes, especially aneuploidy, and where normality is based essentially on the nontumorigenic capacity of the cells. Whether such cells retain the ability to fulfil their genetically programmed functional state *in vivo* has not been determined. Moreover, although studies using transformed, tumorigenic cells (Friend leukaemia[1], myeloid leukaemia[2] and neuroblastoma[3]) have given useful information on some of the factors involved in differentiation, the fact that the cells are tumorigenic indicates that caution must be exercised in the interpretation of results. We report that we have now isolated and cloned an autonomous haematopoietic cell line where the cells have a diploid complement of chromosomes, are non-tumorigenic and bipotential (can be induced to differentiate *in vivo* into two distinct haematopoietic lineages), and which in appropriate circumstances protect mice from potentially lethal radiation.

The cell line was isolated from a long-term culture[4] of (C57BL/6 × DBA/2)F_1 female bone marrow cells, which had been infected with NB-tropic, polycythaemia-inducing Friend

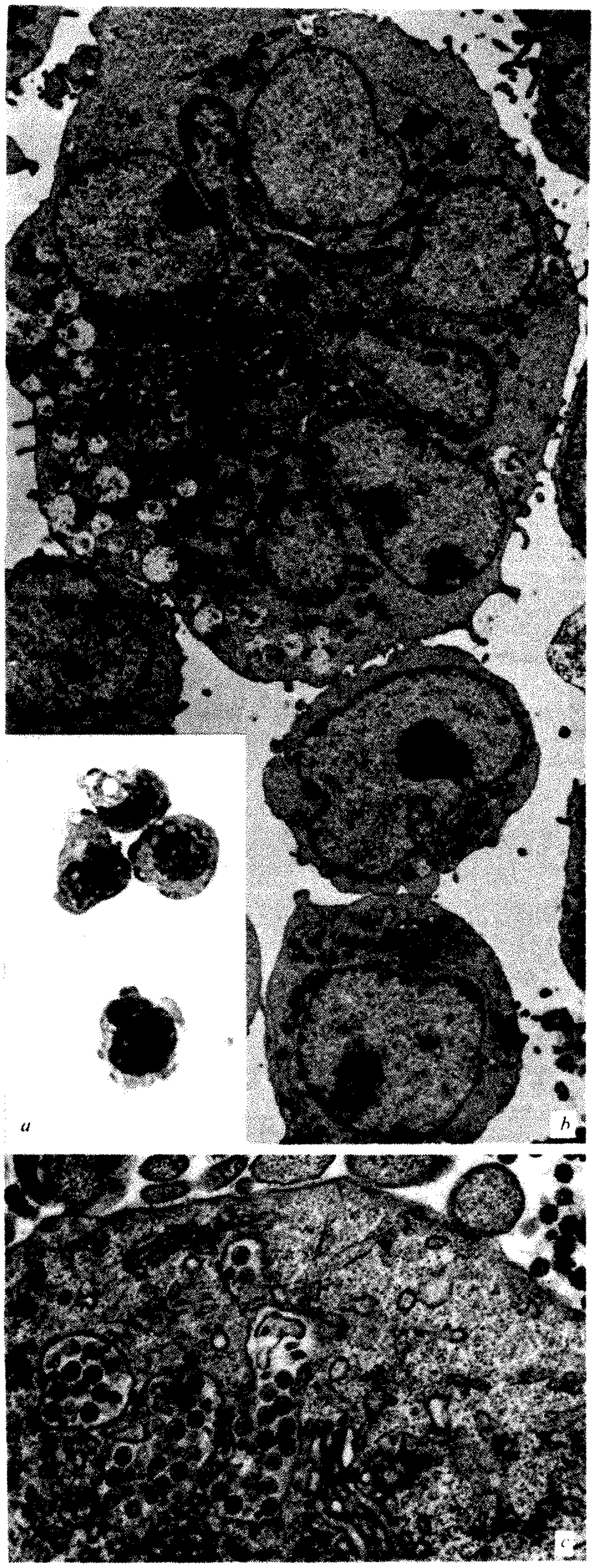

Fig. 1 *a*, Cultured 416B cells stained with May–Grunwald Giemsa. Note large nuclear:cytoplasmic ratio, vacuolated cytoplasm (×750) *b*, Section through a pellet of 416B cells after glutaraldehyde–osmium fixation, showing the size variation of cultured cells. Three small round cells are apparent (corresponding to *a*), with smooth nuclear profiles and undifferentiated cytoplasmic morphology. The large cell (perhaps a megakaryocyte precursor) is characterised by size, dense mitochondrial population and extensive nuclear segmentation (×4,500). *c*, Detail of the cytoplasm of an undifferentiated cell, showing vacuoles containing intracellular immature virus particles, lacking the external coat. Extracellular virus particles are also apparent (×26,000).

leukaemia virus (FLV)[5]. Table 1 shows the essential features of these cultures. For the first 5–6 weeks, the virus-infected cultures behaved similarly to the controls, with both groups demonstrating extensive production of granulocytes (all stages of maturation) and maintenance of haematopoietic stem cells (CFU-S)[6] and granulocyte precursor cells (CFU-C)[7,8]. The CFU-S formed spleen colonies containing all haematopoietic elements (erythroid, granulocytic and megakaryocytic). Subsequently, haematopoiesis in the control cultures decreased, and eventually only phagocytic mononuclear cells remained. In the virus-infected cultures, on the other hand, there was a sustained granulopoiesis (with a marked shift towards the production of immature forms) and a massive increase in CFU-S. However, as previously reported[5], such CFU-S show limited differentiation capacity, producing spleen colonies containing only granulocytes and megakaryocytes. No erythropoiesis was observed. It is particularly significant that throughout the culture period, injection of the culture cells or cell-free supernatants into neonatal or adult syngeneic mice did not result in the development of leukaemia at >1 yr post-inoculation. In this respect, therefore, the cultures differ from our previously reported results[5] where we showed that following infection of genetically susceptible marrow cultures with FLV complex, both the helper (LLV) and the 'defective' (SFFV) viral components replicated, resulting in a maintenance of the erythroleukaemia-inducing capacity of the virus. In the present experiment, although the LLV component is detectable (with weekly titres

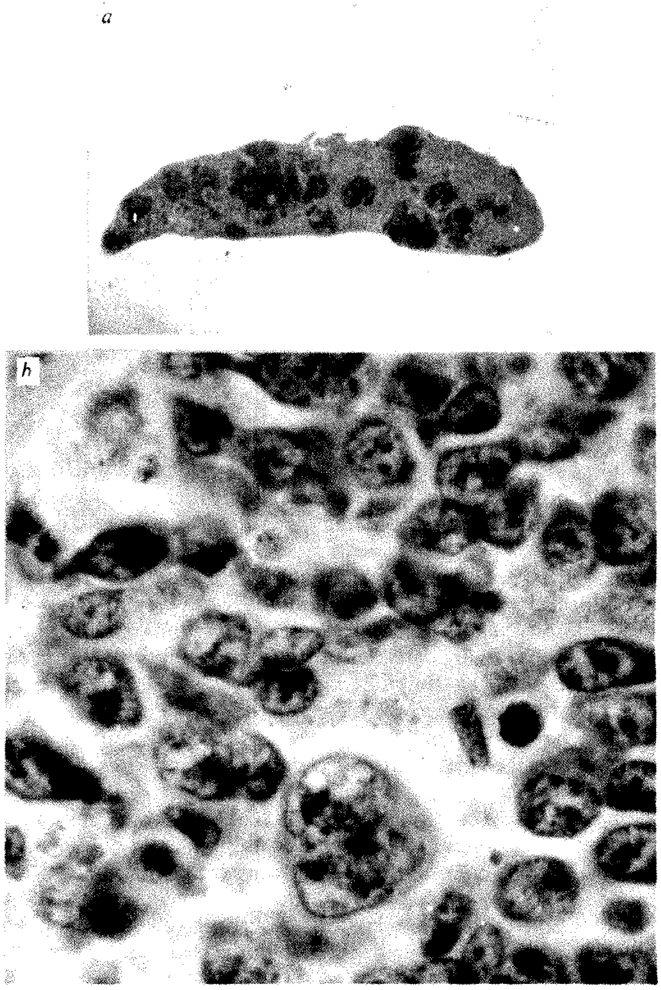

Fig. 2 *a*, Section through mouse spleen (stained with haematoxylin and eosin) showing colonies produced from 416B cells (×5). *b*, Part of a spleen colony, demonstrating granulocytes in various stages of maturation and a single megakaryocyte (×750).

Table 1 Characteristics of normal and Friend leukaemia virus-treated long-term marrow cultures

Weeks cultured	Cell count $\times 10^5$	Morphology B	EG	LG	Mono	Total CFU-S	Total CFU-C
1	14.8(11.3)*	4(0)	8(4)	58(62)	30(32)	703(358)	ND(4,380)
5	20.4(54.0)	35(27)	29(30)	23(36)	10(8)	340(1,120)	5,050(18,000)
10	20.8(10.2)	10(3)	70(8)	5(60)	12(28)	1,730(211)	2,670(ND)
14	21.6(6.6)	30(8)	60(10)	1(6)	5(76)	32,940†(0)	ND(328)
16	31.8(1.0)	16(0)	66(0)	1(2)	15(98)	136,740†(0)	ND(0)
18	33.6(3.0)	25(0)	70(0)	0(0)	4(100)	500,000†(0)	ND(0)
20	50.0(1.8)	40(0)	55(0)	0(0)	5(100)	250,000†(0)	>50,000†(ND)

Cultures were established as previously described[5]. The cells were tested weekly for CFU-S and/or CFU-C and smears made for morphological examination. B, Blast cells, EG, promyelocytes and myelocytes; LG, metamyelocytes and polymorphonuclear cells; Mono, phagocytic mononuclear cells; ND, not done.

* Control (normal) values are in parentheses.

† Restricted differentiation capacity.

ranging from 2×10^4 to 8×10^5 plaque-forming units per ml (ref. 9), the erythroleukaemogenic ability (the SFFV component) has been lost. The precise reasons for this are unclear (and now under investigation), but the end result was that we were able to isolate a cell line with unique characteristics.

After 20 weeks of culture (Table 1), aliquots of cells were transferred to fresh culture flasks. After several days, cell proliferation of a nonadherent population was observed and further sub-culture was possible. The cells were designated 416B and samples of these early isolates were cryopreserved. Morphologically, the cells are heterogeneous: the majority are undifferentiated blast cells and 'promyelocytic' cells (Fig. 1*a*), with occasional larger cells with lobed nuclei. Ultrastructural analysis shows that the majority of cells are undifferentiated, have a large nuclear : cytoplasm ratio, smooth nuclear profiles and limited condensed chromatin (Fig. 1*b*). A minor proportion of cells shows some nuclear segmentation characteristic of immature granulocytes. The larger multi-lobed cells strongly resemble megakaryocytes (Fig. 1*b*). Many cells show cytoplasmic and extracellular forms resembling C-type virus particles (Fig. 1*c*). The population doubling time of the cells is 38–44 h. Karyotype analysis showed that about 95% of the cells possess a diploid chromosome complement, the remaining cells being true tetraploid or octaploid cells; this supports the proposal that the larger cells are developing megakaryocytes.

The colony-forming ability of the cells *in vitro* is shown in Table 2. Cells were plated in the presence or absence of heart conditioned medium[10] as a source of colony-stimulating activity (CSA) which is essential for the growth of normal bone marrow granulocyte precursor cells (CFU-C)[11]. At low cell concentration, colony formation occurred only in the presence of CSA-containing medium, but at higher cell concentrations, CSA was not necessary. Thus, at high cell densities, the cells apparently produce growth-promoting factors; it is not known whether such factors are equivalent to CSA. The colonies consisted of loosely dispersed cells, reminiscent of 'macrophage' colonies[11], and in this respect differed from the typical compact colonies generally formed by leukaemic murine cells. Morphological analysis of the colony cells showed them to be similar to the parent population, with no obvious differentiation. Individual colonies were isolated from soft agar; all such isolates readily proliferated and produced permanently growing cell lines with characteristics identical to the original parental population, including the cell heterogeneity previously mentioned. We have tried to induce *in vitro* differentiation and maturation of the cells, using a variety of agents that promote differentiation of certain granulocytic or erythroid leukaemia cell lines[1,2]. These included purified L-cell CSA, purified erythropoietin, dimethyl sulphoxide, 5-bromodeoxyuridine, dexamethasone, and conditioned medium obtained from long-term cultures of normal mouse bone marrow cells. None of these reagents has so far induced morphological differentiation.

The cells do, however, form macroscopically visible colonies in the spleens of irradiated mice (Table 2 and Fig. 2*a*); although the number produced varied widely, on all occasions the colonies contained only granulocytes and megakaryocytes at all stages of maturation (Fig. 2*b*). Smears taken from the bone marrow also showed this limited differentiation pattern (Fig. 3*a*, *b*). There was no evidence of erythropoiesis or lymphopoiesis. Thus, in the appropriate *in vivo* environment, the cultured cells undergo apparently normal development. However, mice reconstituted with the cultured cells rapidly became anaemic due to defective erythropoiesis, and death occurred 11–20 d post-irradiation. In an attempt to prolong survival, the reconstituted mice were given weekly intraperitoneal injections of 0.5 ml packed red blood cells; 30% of these mice survived for longer than 7 weeks. After 2 weeks only granulocytic and occasional megakaryocytic cells were found. Surface marker analysis failed to demonstrate the presence of Thy-1 antigen or surface immunoglobulin-positive lymphoid cells.

Table 2 *In vitro* and *in vivo* colony formation by 416B cells

In vitro			*In vivo*			
Cells plated (3 ml)	CSA Present	Absent	Cells injected	Spleen colonies I	II	III
10^3	15±2	0	5×10^2	20±1	0	0
10^4	182±21	0	10^3	28±3	0	0
10^5	>1,000	>1,000	5×10^3	Confluent	1	0
5×10^5	Confluent colonies	Confluent colonies	10^4	Confluent	2.5	1
			10^5	Confluent	25.3	18.5

In *in vitro* experiments, the cells were plated in 3 ml Fischers medium supplemented with 20% horse serum (Flow) and antibiotics, in 0.3% agar. CSA was added as a 20% mouse heart conditioned medium[10]. Results are the mean and standard error of three experiments. In *in vivo* experiments, the cells were injected into mice previously exposed to 800 rads. The animals were killed 8–9 d later and the spleens removed and fixed in Bouin's solution. Individual colonies were counted with a dissecting microscope. Experiments I, II and III were carried out 2, 5 and 10 months, respectively, after establishing the cell line.

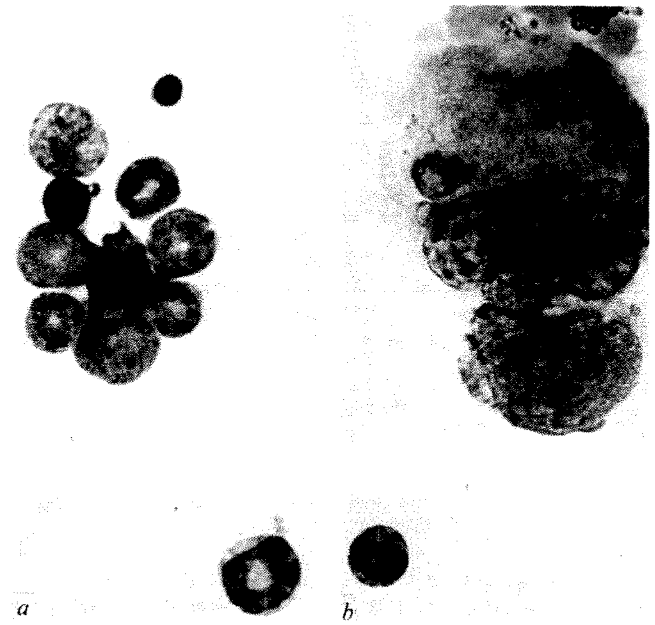

Fig. 3 *a*, Bone marrow taken from mice 2 weeks after injection of 416B cells; note extensive granulopiesis (×750). *b*, As *a*, showing megakaryopoiesis (×750).

At 6 weeks post-reconstitution, spleen and bone marrow cellularity had recovered to near normal values. Within the spleen the granulocytes were the major population, although 14% of the cells were Thy-1-positive lymphocytes; B cells were absent. Granulocytes were also found in the bone marrow and at this time limited erythropoiesis was apparent. Karyotyping of the cells showed that approximately 50% of the spleen cells were of donor origin, whereas in the bone marrow, all the cells were of host origin. This presumably represents re-emergence of a surviving host stem cell population[12] to provide erythropoiesis and T-lymphopoiesis. This finding is not surprising, as the elegant experiments of Micklem *et al.*[13,14] showed that stem cells differ in their 'competitiveness'. For example, when irradiated mice are reconstituted with a mixture of stem cells derived from the peripheral blood and bone marrow, both populations can initiate haematopoiesis, and presumably both can contribute to the 'short-term' survival of these animals. Within a few weeks, however, the number of 'blood-derived' cells declines and the haematopoietic system is reconstituted with marrow-derived stem cells[14]. That system seems analogous to the one reported here and similar mechanisms may be in operation. Indeed, there is increasing evidence to indicate that there is a hierarchy of stem cells (CFU-S) which differ in self-renewal capacity[12-15] and perhaps in their ability to differentiate. The cell line described here may have arisen from a cell with restricted differentiation potential. The relatively few long-term survivors in our reconstitution system may be related to the delayed onset of lymphopoiesis and consequent severe immunodeficiency in these animals.

Several questions arise. (1) Can the differentiation 'block' be overcome *in vitro*? (2) Is there a sub-population of normal stem cells *in vivo* which has similarly restricted differentiation potential, or is the restriction imposed on the cells following infection with leukaemia virus? (3) Will antisera raised against the cells determine new haematopoietic or differentiation linked antigens? (4) Can the cells be induced to undergo leukaemic transformation and, if so, do specific chromosomal changes[16] occur? The answers to these points will give us valuable information on the nature of haematopoietic cell differentiation and transformation.

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A specific helper factor which enhances the cytotoxic response to a syngeneic tumour

IMMUNE RESPONSES are regulated at least in part by a complex system of cellular interactions which result in the specific enhancement or suppression of the reaction to a given antigen. In many situations soluble 'factors' seem to be able to replace these cells, although whether they actually do so *in vivo* remains unknown. Specific suppressor factors have been demonstrated to influence a variety of humoral and cell-mediated immune responses. Specific helper factors which augment antibody production have been studied extensively[1-3], but analogous factors active in cell-mediated immunity have not yet been documented. Soluble factors which enhance the generation of cytotoxicity have been reported by several workers[4-8] but none of these has been demonstrated to be antigen specific. We report here the isolation of a helper factor which specifically enhances the generation of cytotoxic cells in a syngeneic mouse tumour system.

We have shown previously that cells from spleen or thymus of mice bearing the P815 mastocytoma, or extracts from these organs, can specifically suppress the generation *in vitro* of cells cytotoxic to P815 (refs 9–11). These results are in basic agreement with those of Fujimoto *et al.*[12] and Green *et al.*[13], who examined the influence of suppressor cells or factor on the *in vivo* growth of methylcholanthrene-induced tumours. In either assay system the activity measured is a net value which could comprise components of both help and suppression. Fractionation of our crude thymus suppressor factor by isoelectric focusing or by gel filtration indicated that there were fractions showing net enhancement of cytotoxicity suggesting the presence of both a helper and a suppressor factor in the same extract. In subsequent work aimed at the isolation of a helper factor, fractions of spleen extracts separated by gel filtration were used.

Figure 1 shows the effluent profile of extracts from normal or 8-d tumour-bearer spleens separated on G150 Sephadex. The

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fractions were added to cultures containing normal DBA/2 spleen cells together with mitomycin-C-treated P815 cells as antigen. After 5 d the cells were collected and tested for cytotoxicity against P815 in an overnight ^{51}Cr-release assay. Results from cultures containing fractions from the tumour bearers were compared with those from cultures containing an equivalent concentration of normal fractions. Only fractions II and III showed significant differences between the tumour and normal extract. Fractions prepared from extracts of spleens taken at various times after tumour injection indicated that suppressive activity, if present, was in fraction II, whereas enhancing activity appeared in fraction III, indicating that the two activities could be at least partially separated on the basis of size (these data will be presented elsewhere). For subsequent work on the purification of a helper factor, fraction III from 8-d tumour-bearer spleens was used.

Anti-P815 cytotoxicity generated in cultures containing tumour-bearer fraction III (TIII) at concentrations between 0.1 and 0.05 spleen equivalents was consistently enhanced (five experiments, data not shown), as compared with controls containing equivalent amounts of normal factor III (NIII). Further purification of the factor was affected by absorbing NIII or TIII on to columns of P815 membrane fragments linked to Sepharose. Bound material was eluted in mildly dissociating conditions with 2 M salt. As shown in Fig. 2*a*, the eluted material derived from TIII was markedly enhancing at a concentration of 0.05 spleen equivalents. Equivalent results were obtained with 0.005 spleen equivalents, but at lower concentrations than this the helper activity was diluted out (data not shown). However, the control eluate derived from NIII did not influence the response at any concentration tested. The data in Fig. 2*a* show that the purified helper factor increased the cytotoxicity about fourfold in terms of cytotoxic units. The specificity of the factor was tested by adding it to parallel cultures containing mitomycin-treated L1210 rather than P815 as antigen. Neither fraction had any effect on the generation of anti-L1210 cytotoxicity (Fig. 2*b*). Similarly, the P815 helper factor did not enhance anti-H-2^d killing generated in cultures of C57BL/6 spleen cells with irradiated DBA/2 spleen cells (data not shown). We have shown previously that cytotoxicity in the syngeneic system is T-cell dependent (and probably T-cell mediated), in that treatment of the effector population with anti Thy-1 antiserum and complement before the 18-h abolishes killing[10]. This was also true of the enhanced cytotoxicity generated in the presence of P815 helper factor, suggesting that the mechanism of killing was not altered.

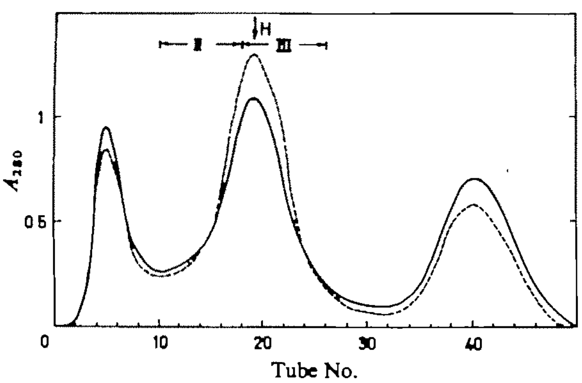

Fig. 1 Fractionation of spleen extracts on Sephadex G150. Extracts were prepared by the technique previously used for thymus tissue[11]. Spleens were taken from 8–12-week-old normal (broken line) or tumour-bearing (solid line) female DBA/2 mice. The tumour bearers were injected 8 d before death with 2×10^3 P815 mastocytoma cells in the right flank. At the time of death, the tumour was growing as an easily palpable confined mass at the site of injection. There was no evidence of metastasised tumour cells in the spleen by microscopic examination. Five spleens suspended in a small volume of phosphate-buffered saline (PBS) were frozen and thawed, homogenised in a conical glass tissue grinder, sonicated twice for 60 s on ice, centrifuged at 60,000*g* for 60 min at 4 °C, and the supernatant was immediately applied to the Sephadex column. The column was eluted with borate saline, *p*H 8.3, collecting 4-ml fractions which were pooled as indicated. Mouse haemoglobin eluted near the peak of the second peak as indicated by arrow H.

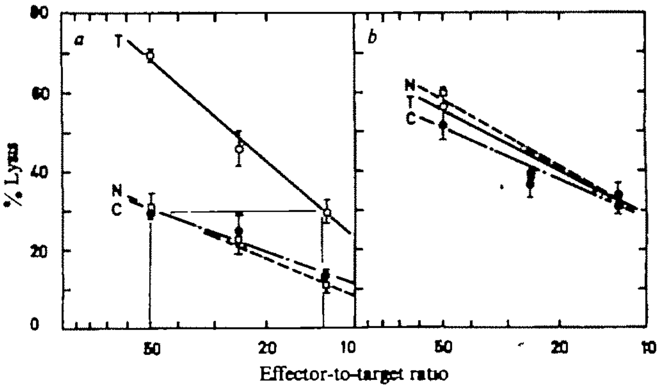

Fig. 2 The effect of P815 membrane column-purified helper factor on the *in vitro* generation of cytotoxicity to syngeneic P815 (*a*) and L1210 (*b*) tumour cells. 5×10^6 normal DBA/2 spleen cells were cultured with 5×10^5 mitomycin-C-treated P815 or L1210 cells in 2.5 ml of RPMI 1640 in 24 well Linbro plates (No. 76–033–05). Cultures contained 10% fetal calf serum, 10 mM HEPES, 2×10^{-5} M 2-mercaptoethanol, with or without 0.05 spleen equivalents of test fractions. After incubation for 5 d at 37 °C the cells were collected and set up in a standard 18-h ^{51}Cr-release assay[9] against ^{51}Cr-labelled P815 or L1210. The results show the averages and standard deviations of triplicate cultures. The P815 membrane Sepharose column was prepared as previously described[11] by linking a sonicate of P815 membranes obtained by hypotonic lysis to Sepharose 4B activated with cyanogen bromide. Unreacted groups were blocked with ethanolamine and the absorbent was washed with 0.1 M HCl and 2 M NaCl before use. Fractions were stirred for 15 min at either 4 °C or room temperature with the absorbent. The absorbent was then poured into a small column, washed with PBS until the A_{280} of the eluate was <0.01 and then eluted with 2 column volumes of 2 M NaCl. The A_{280} of the eluted material was in all cases <0.01. It was stored at −70 °C. Concentration is quoted as spleen equivalents based on the volume of original spleen homogenate, corrected for the change in volume following gel and affinity chromatography. Values for the eluted fractions are maximums based on the assumption that all the activity in the Sephadex fraction was recovered. Identical results were obtained using 0.005 spleen equivalents of purified factor. It can be seen in (*a*) that for an equivalent level of cytotoxicity, for example, 30%, about a quarter of the number of cells from helper-induced cultures were required as compared to controls, indicating an approximately fourfold increase in cytolytic units. ○, □, Cultures containing eluted fractions derived from TIII or NIII, respectively; ●, control cultures without added factor.

Helper factors in other systems[2,14,15] have been shown to be coded for by the I region of the major histocompatibility complex (MHC). Figure 3 indicates that this is also true for the P815 helper factor. In this experiment, the factor was incubated overnight with the antiserum; any immune complexes as well as residual free mouse immunoglobulin (Ig) were then removed on an anti-mouse Ig immunoadsorbent column. The capacity of the column was sufficient to bind twice the amount in the samples. Thus, free antibody, which might influence the response, was not added to the cultures.

The anti Ia used here was prepared by immunising B10 mice repeatedly with B10D2 splenocytes. To render this serum specific for the Ia region of the H-2^d MHC, it was absorbed exhaustively against P815 cells until it was no longer cytotoxic for these targets in the presence of rabbit complement. The properties of this absorbed antiserum are outlined elsewhere[16]. It can be seen that helper activity was absorbed by this antiserum. As controls, either anti H-2^k or normal B10 serum absorbed with P815 (results not shown) were used. The purpose

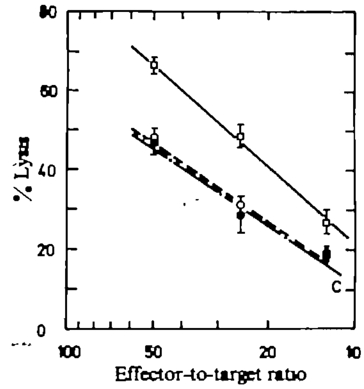

Fig. 3 The effect of anti-Ia antiserum absorption on the activity of P815 membrane column-purified helper factor. The ability of anti-Ia^d to remove helper activity from purified helper factor TIIIe was tested by an immunoadsorbent assay described previously[11]. Anti-Ia^d antiserum was prepared from B10 anti-B10.D2 antiserum by absorption with P815 as outlined elsewhere[16]. The control antiserum was B10.D2 anti-B10.BR. The antiserum and factor were incubated overnight at 4 °C and then passed over an immunoadsorbent column of goat anti-mouse Ig. This material was then added to cultures at a concentration of 0.01 spleen equivalents. Identical results were obtained in a second experiment using P815-absorbed normal B10 serum as the control. □, anti H–2^k; ○, anti Ia^d; ●, control.

of these controls was to show that the loss of helper activity was not due to some inhibitory effect of anti-H–2 antiserum or to the presence of P815 antigen which might combine with the helper factor. In neither case did the control antiserum remove activity. The results of this experiment show that the helper factor bears I-region-coded markers but does not possess constant-region determinants of mouse Ig. They do not exclude the possibility that the factor also has K^d or D^d determinants.

The properties of the P815 helper factor are consistent with those of helper factors active in the antibody response. It binds antigen, is not a conventional antibody molecule, and seems to be I-region coded. Its molecular weight is about 50,000 by gel filtration. Preliminary results from SDS-polyacrylamide gel electrophoresis of iodinated factor in reducing conditions indicate that it is a monomer of MW 65,000. We know of no other reports of antigen-specific helper factors active in the generation of cytotoxic T cells . There is, however, recent evidence of a requirement for specific helper cells in the generation of killer T cells in allogeneic systems.[17] As well as providing an additional way to study the mechanisms that regulate the generation of cytotoxic cells, the factor described here may offer a means of altering the course of tumour growth *in vivo*.

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Cytolytic and proliferative activity of a permanent T killer cell line

ATTEMPTS to establish lymphocyte cell lines with specific cytotoxic activity by viral transformation of cytotoxic thymus-derived lymphocytes (T killer cells) have been unsuccessful in many laboratories. Screening of thymic lymphomas for specific cytolytic activity and attempts to hybridise thymic lymphomas with T killer cells have also been unsuccessful. During these studies it has become apparent, however, that alloreactive T cells derived from a primary mixed lymphocyte culture can be maintained in tissue culture for several months or even years, provided these cells are periodically stimulated with irradiated allogeneic spleen cells[1,2]. Many of these cell lines, although retaining specific proliferative activity, tend to lose their cytolytic activity after about 6–12 months[1–4]. This, of course, makes these cell lines less useful for studying the receptor specificity, cytolytic activity or clinical usefulness of T killer cells. Here I report the successful establishment in tissue culture of a cell line which has retained both specific proliferative and cytotoxic activity for almost three years in continuous culture.

Table 1 Cytolytic activity of C C3 11 · 75

	Target*	H–2	Attacker to target ratio	PHA†	% Cytotoxicity‡
					5 H
Expt 1	C3H blasts	kkkkkkk	30·1	–	37±3
	C3H blasts	kkkkkkk	10.1	–	19±1
	R1(TL$^+$)	k . . . k	30·1	–	<1
	R1(TL$^+$)	k . . k	10:1	–	<1
					3 h
Expt 2	C3H blasts	kkkkkkk	50·1	–	37±2.4
	C3H blasts	kkkkkkk	15:1	–	29±2.2
	BALB/c blasts	ddddddd	50·1	–	<1
	BALB/c blasts	ddddddd	15·1	–	<1
	C3H blasts	kkkkkkk	50:1	+	43±0.5
	C3H blasts	kkkkkkk	15:1	+	37±0.9
	BALB/c blasts	ddddddd	50 1	+	45±0.2
	BALB/c blasts	ddddddd	15.1	+	36±0.9
					3 h
Expt 3	C3H blasts	kkkkkkk	50:1	–	40±2.5
	B10A blast	kkkdddd	50:1	–	43±1.1
	B10A (4R) blasts	kkddddd	50 1	–	42±0.8
	ATL blasts	skkkkkd	50 1	–	39±1.1
	C3H OL blasts	ddddkkk	50:1	–	4±2.7
	BALB/c blasts	ddddddd	50·1	–	<1

* Spleen cells (10^6 per ml) were cultured for 3 d in the presence of 10 μg ml^{-1} bacterial lipopolysaccharide, collected and labelled with ^{51}Cr (ref. 2).

† PHA concentration was 5 μg ml^{-1} in the assay.

‡ The following equation was used to express cytotoxicity:

$$\% \text{ cytotoxicity} = \frac{(\text{experimental } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})}{(\text{maximal } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}) \times 100}$$

Maximal release was calculated by freezing and thawing the target cells three times. Values are means of duplicates Spontaneous release of targets was as follows expt 1: C3H = 5.1% per h, R 1(TL$^+$) = 2.5% per h. Expt 2. C3H = 4.7% per h; BALB/c = 5.2% per h. Expt 3· C3H = 4.8% per h, B10A = 5.1% per h, B10A(4R) = 4.5% per h; ATL = 4.7% per h; C3H 0L = 3.4% per h, BALB/c = 5.2% per h.

Mixed lymphocyte cultures containing 10^5 per ml responder BALB/c spleen cells and 10^5 per ml 1,000 rad irradiated C3H stimulator cells were set up in Click's minimal essential medium supplemented with 5% selected fetal calf serum, 5×10^{-5} M mercaptoethanol, antibiotics and glutamine in 20 ml Falcon no. 3013 tissue culture bottles[1,2]. Culture flasks were incubated at an upright position in 5% CO_2 in air at 37 °C. Every 2 weeks the

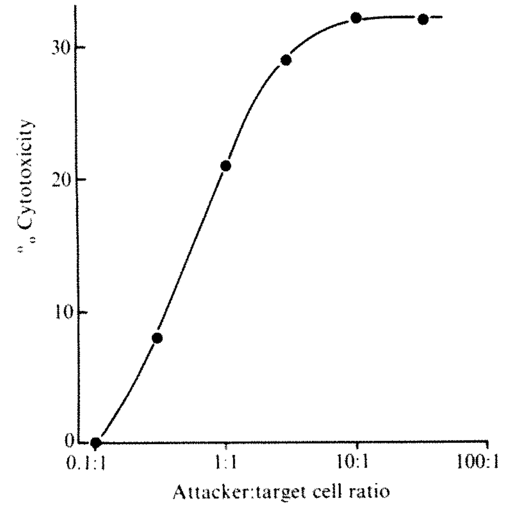

Fig. 1 C · C3 · 11 · 75 was titrated for its cytotoxic activity on C3H blast cells at various attacker to target cell ratios. The 3-h time point is given. Spontaneous release of C3H targets was 4.3% per h.

culture supernatant was replaced with new medium containing 10^5 per ml irradiated C3H spleen cells. The culture was regularly split when the cell density reached 5×10^5 cells per ml. The cell culture was called C·C3·11·75 as it is a BALB/c (H-2^d) anti C3H (H-2^k) T killer culture which was originally set up in November 1975 (ref. 2). After 5 months in culture C · C3 · 11 · 75 showed strong cell proliferation—about 10-fold over background—when challenged with C3H (H-2^k), CBA (H-2^k) and A (H-2^a) spleen cells but little or no stimulation was caused by DBA/1 (H-2^q), C57BL/6 (H-2^b), ASW (H-2^s) or BALB/c (H-2^d) spleen cells[2]. Strong cytotoxic activity was assayed on H-2^k spleen blast cells or tumour cells[2]. Beginning at about 10 months the cytolytic activity of C · C3 · 11 · 75 as assayed on tumour targets gradually disappeared as previously seen with other T killer cell lines[1–3]. However, as shown in Table 1, expt 1, cytolytic activity to C3H spleen blast cells is retained, while R1(TL$^+$) which expresses both the K end private specificity H-2.23 and the D end private specificity H-2.32 (ref. 5) is not lysed. Syngeneic BALB/c spleen blast cells are only lysed in the presence of phytohaemagglutinin (PHA) which only slightly increases cytotoxicity on C3H spleen cell blasts (Table 1, expt 2). The failure of C · C3 · 11 · 75 to lyse R1(TL$^+$) suggested that this killer cell line recognises other than the K or D region coded antigens. In support of this, Table 1 expt 3 shows that not only C3H, but also B10A, B10A (4R) and ATL, spleen blast cells are lysed. In contrast, C3H OL and BALB/c blast cells are not lysed. Taking the failure of C · C3 · 11 · 75 to lyse R1(TL$^+$) into account this would suggest that the determinants recognised by this cell line are encoded by genes to the right of the K region, that is, in the IA subregion[6]. As genes in this region are responsible for T-cell proliferation it was tested whether the spleen cells which are susceptible to lysis by C · C3 · 11 · 75 are able to stimulate cell proliferation. It can be seen from Table 1 that C3H OL does not stimulate cell proliferation, whereas BALB/c shows consistently significant stimulation. It is not clear what the basis of this stimulation might be. Cloning of this cell line will resolve the question of whether this cell line contains alloreactive as well as autoreactive cells. Aside from the stimulation by BALB/c much higher stimulation is seen with C3H, B10A and ATL spleen cells, which are good targets in the cytotoxic reaction. It therefore seems that the original population of killer cells was heterogeneous, consisting of cells which recognise several specificities expressed on both tumour cells and spleen blast cells. Prolonged selection of this cell line by periodic stimulation with C3H spleen cells has resulted in a population which only kills targets expressing antigens coded by the IA subregion genes[6]. It is possible but not certain that these same antigens are responsible for the proliferation of this cell line. C · C3 · 11 · 75 is sensitive to anti-θ and complement lysis and is also lysed by an anti H-2^d serum in the presence of complement. It can be frozen in liquid nitrogen and will start to proliferate after thawing with a doubling time of ~45 h. Both its proliferation and cytolytic activity depend on weekly stimulation with C3H spleen cells. A titration curve of its cytotoxic activity is shown in Fig. 1. Interestingly, the cytotoxicity has reached plateau values at attacker to targer ratios lower than 10:1. This supports the contention that C · C3 · 11 · 75 is a rather homogeneous T killer cell population. Maximal cytolysis is always found to be in the range 30–45% which may be because of heterogeneity in the target population or inhibition of the effector cells by soluble antigen.

Table 2 Proliferative activity of C · C3 · 11 · 75

Responder*	Stimulator	H-2	Proliferative activity† (c.p.m.)
5×10^4 C · C3 · 11 · 75	—	—	68 ± 13
—	BALB/c	ddddddd	77 ± 4
—	C3H 0L	ddddkkk	115 ± 15
—	C3H	kkkkkkk	100 ± 3
—	B10A	kkkdddd	77 ± 7
—	ATL	skkkkkd	76 ± 3
5×10^4 C · C3 · 11 · 75	BALB/c	ddddddd	1,590 ± 192
5×10^4 C · C3 · 11 · 75	C3H 0L	ddddkkk	168 ± 16
5×10^4 C · C3 · 11 · 75	C3H	kkkkkkk	12,138 ± 2,057
5×10^4 C · C3 · 11 · 75	B10A	kkkdddd	8,514 ± 723
5×10^4 C · C3 · 11 · 75	ATL	skkkkkd	5,802 ± 118
5×10^4 C · C3 · 11 · 75	Con A supernatant‡	—	137 ± 22

* 5×10^4 C · C3 · 11 · 75 were cultured with 5×10^6 per ml 1,000-rad irradiated stimulator spleen cells in 1-ml tubes in RPMI-1640 medium (ref. 2).

† Cultures were labelled with 0.5 µl ^{3}H-thymidine on day 2 and collected on day 3 (ref. 2).

‡ Con A supernatant was prepared by culturing mouse spleen cells with Con A for 2 days. The supernatant was filtered and tested as described in refs 7 and 8.

Recently it was reported that alloreactive killer cells can be propagated up to 5–7 months by weekly stimulation with supernatants of spleen cells stimulated with concanavalin A (Con A)[7,8]. It is likely that these cells which can be stimulated by Con A supernatant are quite different from those described here[8]. One important difference is that C · C3 · 11 · 75 cannot be stimulated by Con A supernatants (Table 2) but by spleen cells while the converse is true for the killer cells described by Nabholz *et al.*[8]. The establishment of an apparently continuously proliferating cytotoxic T-cell line which has retained specific biological functions may be the ideal tool to study the cell surface receptors and the function of this important class of lymphocytes.

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An Lyt differentiated thymocyte subpopulation detected by flow microfluorometry

DATA on the T-cell antigens, Lyt 1, 2, 3 (refs 1, 2), relating to functional T-cell subsets, suggest that some peripheral T cells express restricted Lyt phenotypes, that is, Lyt 1^+23^- or Lyt 1^-23^+ (ref. 3). Evidence has been presented showing that two major subpopulations of lymphocytes, Lyt 1^+ T cells and Lyt 23^+ T cells, are committed to particular lines of functional differentiation in the immune response before encountering exogenous antigen in peripheral lymphoid tissue[4,5]. It has been suggested that the two major subpopulations representing T-cell help (Lyt 1^+) and T-cell cytotoxicity or suppression (Lyt 23^+) arise in the periphery from peripheral Lyt 123^+ cells[6]. Recent studies have concluded that as stem cells mature in the thymus, the thymic epithelium is important in determining which H-2 specificities the maturing T cells are able to recognise as self[7]. Previous cytotoxicity data have been interpreted as indicating that Lyt 1, 2, 3 antigens are expressed on each of most if not all, mouse thymocytes; that is, that most thymocytes are Lyt 123^+ (refs 1–4, 8–10). However, the data presented here suggest that the commitment to differentiation of the Lyt 1^+ or the Lyt 123^+ phenotype, and thus presumably the relevant functional differentiation occurs before cell migration from the thymus. Using flow microfluorometry (FMF) and anti-Lyt antisera, we demonstrate a normal thymocyte subpopulation which is not expressing Lyt 2 or Lyt 3 antigens (Lyt 1^+23^-). This subpopulation represents a significant constant proportion (10%) of normal thymocytes which is markedly increased following *in vivo* cortisone treatment. The data also show that Lyt 1 expression, rather than being decreased on cortisone-resistant thymocytes (CRT), as would have been predicted from previous cytotoxicity data[2,8], is expressed in increased amounts on CRT.

We have used thymocytes of C57BL/6 (B6) mice or the appropriate B6/Ly congeneic strains, anti-Lyt antisera and affinity-purified anti-mouse immunoglobulin (Ig) fluorescent staining reagents to re-examine the expression of Lyt 1, 2, 3 antigens on the thymocyte surface. Preparation of these sera and reagents has been described in detail elsewhere[11–13]. Immunofluorescence was analysed by FMF[14] on a FACS II interfaced to a PDP 11/40 computer[15]. This allowed us to quantitate total cell surface immunofluorescence on individual cells.

Figure 1*a*–*d* indicates that both anti-Lyt 1.1 and anti-Lyt 1.2 react with >99% of all thymocytes. The Lyt 1 expression for both alleles is increased on CRT (Fig. 1*b*, *d*) relative to normal thymocytes (Fig. 1*a*, *c*), as indicated by the increased level of fluorescence on Lyt 1^+ cells; for example, 50% of CRT have >1,200 fluorescent units per cell compared with 19% of normal thymocytes (Fig. 1*a*, *b*). In contrast, anti-Lyt 2.1 and anti-Lyt 2.2 (Fig. 1*e*–*h*) react with only 90% of normal thymocytes and either allele is expressed on only 30–50% of CRT. When the Lyt 2 reagents were used at saturation, the mean peak of fluorescence of the positive cells on the cell frequency distribution histogram indicated that Lyt 2^+ CRT cells were expressing less detectable antigen on the surface than normal thymocytes. Normal B6 thymocytes from neonates are similar to those from adults in Lyt expression, indicating a constant 10% Lyt 2^- thymic subpopulation at least from birth.

There is an apparent correlation between the expression of Lyt 3 and that of Lyt 2. (1) The same number of normal

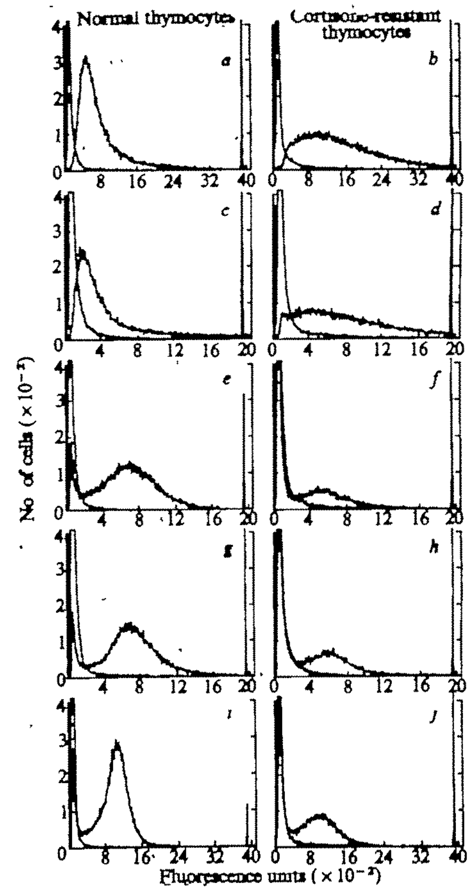

Fig. 1 Normal or cortisone-resistant thymocytes from 3–5-month C57BL/6 (B6) or appropriate B6/Ly congeneic mice were treated with anti-Lyt antisera and fluorescent goat anti-mouse immunoglobulin (Fl-GAMIg). CRT were obtained 36–40 h after intraperitoneal administration of 0.25 mg cortisone acetate in suspension. For each analysis, 10^6 cells, suspended in phenol red-free HBSS modified with 0.1% NaN_3 and 0.1% bovine serum albumin were incubated for 45 min at 4 °C with antiserum and washed twice by centrifugation in 2 ml volumes of the same medium. The anti-Lyt-sensitised cell pellets were then resuspended in about 50 µl residual liquid, incubated for 30 min with Fl-GAMIg, washed twice, resuspended in 0.4 ml of modified HBSS, and analysed by FMF on a FACS II (Becton Dickinson) which is equipped with an argon ion laser operated at 488 nm wavelength and 200 mW for fluorescein excitation. The beam is focused by a 39-mm focal length spherical lens. Fluorescence is detected with an S-11 response photomultiplier tube at 520 V with 520-nm and 530-nm barrier filters. Each section of the Figure displays two cell frequency histograms, positive thymocytes (normal or CRT) and the appropriate negative control cells (normal or CRT), treated equally with the same antiserum and fluorescent reagent. Each histogram represents the fluorescent staining intensities of 50,000 viable cells; viability determined by light scatter[14]. Data were collected on the FACS II in a 1,000 channel distribution and stored in a PDP 11/40 computer (Digital Equipment Corporation) interfaced to the FACS II (ref. 15). Fluorescence unit values have been corrected for differing amounts of amplification used when collecting fluorescence signals from cells treated with different reagents. *a*,*b*, Anti-Lyt 1.1; positive thymocytes and CRT were obtained from B6/Ly 1.1 mice, control cells were obtained from B6 mice Cell suspensions were incubated with 10 µl of anti-Lyt 1.1 obtained from a pool of multiple bleedings from a single mouse. This pool and ~20 similar pools were obtained from (B6/N × BALB/c AnN)F_1 mice immunised 3–10 times with B6/Ly 1.1 normal thymocytes. All antisera were routinely absorbed with lymphoid cells expressing the opposite Lyt allele, and were centrifuged at 100,000 *g* (30 p.s.i., in a Beckman airfuge) for 20 min to remove complexes and aggregates immediately before use. *c*,*d*, Anti-Lyt 1.2, these cell suspensions were the same as those in *a*,*b*, except that this antiserum recognises the opposite Lyt 1 allele and therefore B6 cells are the positive populations, and B6/Ly 1.1 cells are negative. Anti-Lyt 1.2 was obtained from a pool of C3H/An mice immunised with CE normal thymocytes. 25 µl of this antiserum was used for analysis of Lyt 1.2 expression *e*,*f*, Anti-Lyt 2.1; the positive cell populations in this case were obtained from B6/Ly 2.1, 3.1 mice, and cells from B6/Ly 1.1 mice were used as controls. This antiserum was raised by immunisation of B6/H-2^k mice with CE normal thymocytes. The antiserum was from a highly cytotoxic pool and was also extremely high in anti-Lyt activity measured by this method only 10 µl at 1/10 being needed for these analyses. *g*,*h*, Anti-Lyt 2.2; the positive cell populations were obtained from B6 mice and the negative cell populations from B6/Ly 2.1, 3.1 mice. Anti-Lyt 2.2 anti-serum was obtained as described for the anti-Lyt 1.1 except that (C3H/HeN × B6/Ly 2.1)F_1 mice were immunised with normal B6 thymocytes. This antiserum was used at 15 µl for saturation level of staining analyses. *i*,*j*, Anti-Lyt 3.2; positive cell populations were obtained from B6/Ly 2.1 mice and negative cell populations from B6/Ly2.1, 3 1 mice. Anti-Lyt 3.2 antiserum was made from a pool of immunised mouse sera selected for high levels of cytotoxicity. The mice used for this serum were C58 immunised with CE normal thymocytes. Saturation levels of this antiserum were obtained with 15 µl of 1/10 diluted antiserum.

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thymocytes are positive with reagents detecting either antigen (compare Fig. 1 *e*.*g*.*i*); (2) like Lyt 2 expression on CRT, a higher proportion of Lyt 3^- cells is seen with CRT than with normal thymocytes (compare Fig. 1*i*, *f*); (3) treatment with both anti-Lyt 2 and anti-Lyt 3 still leaves 10% normal thymocytes unstained.

Each anti-Lyt 2 and anti-Lyt 3 histogram shows two nearly symmetrical peaks (Fig. 1*e*-*j*) one of which falls beneath the negative-control histogram peak and contains about 10% of the total cells, the second of which falls almost entirely outside the range of the negative cell control and contains 90% of the total cells. If, in non-saturating conditions, increasing amounts of anti-Lyt 2 are added, the entire dominant positively stained peak of cells is shifted to the right (brighter), whereas the minor peak of negative cells (unstained cells) is unchanged. By contrast, each histogram obtained with anti-Lyt 1.1 or anti-Lyt 1.2 shows one major asymmetrical peak containing >99% of the cells. Although this peak partially overlaps that of the negative control, several lines of evidence indicate that all cells under the major peak are Lyt 1^+: (1) if more anti-Lyt 1 is used, the entire major peak shifts to the right (brighter), such that it no longer overlaps the control peak; (2) the major peak is always unimodal with a trail of bright cells, and (3) a small distinct peak (<0.5% of cells) invariably precedes the major peak and may represent Lyt 1^- thymocytes. In summary, the frequency distribution histograms for normal thymocyte expression of Lyt 1 can be described as a major peak of 'dull' positive cells with a trail of 'bright' cells. In contrast, the Lyt 2.3 histograms can be described as a minor peak of negative cells and a major peak of positive cells with relatively homogeneous staining. The CRT pattern for Lyt 1 is a relatively flat distribution of positive cells with two approximately equal peaks of Lyt 2^+3^+ and Lyt 2^-3^- cells. Thus, the patterns of Lyt 1.1 and Lyt 1.2 antigen expression resemble each other, as do those of Lyt 2.1, Lyt 2.2 and Lyt 3.2. Expression of Lyt 1 antigen is, however, different from Lyt 2 and Lyt 3.

Dual-parameter analysis of forward light scatter (1–15°), a relative measure of thymocyte size[16], and of immunofluorescence for expression of these Lyt antigens was carried out on normal thymocytes and CRT. These analyses indicate that Lyt 23^- cells are medium to large in size and express relatively high amounts of Lyt 1. In contrast, the majority of Lyt 23^+ cells are smaller and express lower amounts of Lyt 1. Figure 2 shows light scatter profiles of B6 CRT showing different staining levels. It is important to remember that fluorescence intensity by this method reflects total antigen expression, not necessarily antigen density.

Recently we described a series of mouse thymic lymphomas which seemed to display limited or restricted Lyt phenotypes similar to functional differentiated, peripheral T cells[13]. Most of the tumours in that study were TL^+. As TL antigen expression is restricted to thymocytes and T-cell lymphomas[17], we suggested that a normal thymocyte subpopulation existed which was TL^+ and already differentiated for Lyt antigen phenotype. Other data[18] also predict the functional differentiation of TL^+ thymocytes. The results presented here confirm the existence of a significant subpopulation of normal thymocytes which are differentiated in expression of Lyt antigens. Similar results have been obtained for several other strains of mice, including BALB/c, A/J, C3H and DBA/2.

The increased expression of Lyt 1 on CRT, as detected by these antisera and FMF, was not predicted by previous cytotoxicity data[4,8]. Increased expression of Lyt 1 cannot be attributed to induction of antigen expression during the tests because the entire procedure was carried out at 4°C in Hanks' balanced salt solution (HBSS) containing 0.1% NaN_3.

The increased expression of Lyt 1 on CRT is probably not attributable to Fc binding of the anti-Lyt antisera because; (1) there are marked fluorescence profile differences between anti-Lyt 1 and anti-Lyt 23 on the same cell suspension (2) appropriate negative congeneic cell controls are not stained in the same conditions with the same antisera (see Fig. 1). (3) staining a 50:50 mixture of cells from mice expressing both alleles (for example, Lyt 1.1 plus Lyt 1.2) yields only 50% positively stained cells (data not shown). This considerably decreases the possibility of activation of Fc regions of Ig leading to nonspecific staining of Lyt 1^- cells. (4) As a precaution, all alloantisera and fluorescent reagents were ultracentrifuged at 100,000 *g* before being used in these tests.

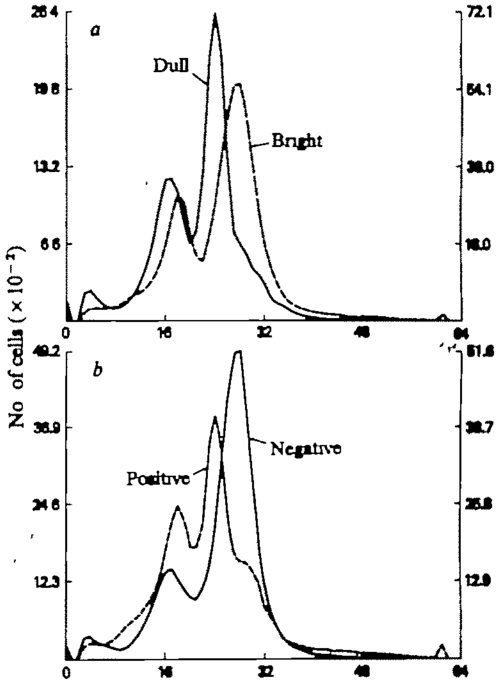

Fig. 2 CRT from C57BL/6 (B6) mice were prepared and treated with anti-Lyt antisera and Fl-GAMIg as described for Fig. 1. FMF analysis was carried out as described in Fig 1, except that data were collected and stored in the computer on both light scatter (LS) and fluorescence (Fl) simultaneously for 10^5 total cells (not selected on LS) Data were collected in a 64 × 64 matrix such that the *x*-axis represented LS, the *y*-axis Fl, and the *z*-axis the cell number at each intersection of LS and Fl. Data collection and analysis are described elsewhere[13]. Here, upper and lower thresholds (windows) were selected on Fl intensity and all LS data within each window are shown as the number of cells versus increasing LS *a*, The LS of B6 CRT staining with different intensities with anti-Lyt 1 2 Dull cells (——) were selected as those with intensities of <384 Fl units (see Fig. 1*d*). This corresponds to the majority of normal thymocytes and 20.6% of viable CRT in this example. The bright (– – –) cells were selected as those with intensities >640 Fl units (see Fig 1*d*) and here represented 65% of the viable CRT The first peak (cells <21 LS units) in all the histograms in this Figure represents dead cells which have lower scatter intensity than viable cells[14], and which in these CRT preparations comprised about 30% of the total cell population. *b* The LS of B6 CRT staining with different intensities with anti-Lyt 2.2. the negative (——) cells were selected as those with intensities <150 FL units (Fig. 1*h*) and represented 51.5% of the viable CRT in this experiment The positive cells (– – –) were selected as those with intensities >260 Fl units (Fig 1*h*) and represented 44.8% of the viable CRT

The discrepancy between complement-mediated, antibody-dependent cytotoxicity and FMF analysis may be explained by one or more of the following. (1) Our technique is more accurate in terms of the ability to measure the quantity of surface antigen expressed on individual cells. Thus, we were able to detect the minor Lyt 1^+, 23^- subpopulation of thymocytes. (2) There might be a quantitative difference in the expression of Lyt 1, and cytotoxicity assays with complement might kill only Lyt 1 'bright' cells. Varying levels of Lyt 1.1 antigen expression on peripheral T cells have already been suggested to explain a titrated depletion of cytotoxic effector T cells with anti-Lyt 1.1[19]. However, other factors are involved, because easily killed normal thymocytes express less Lyt 1 than do CRT by quantitative immunofluorescence (Fig. 1*a*–*d*). Furthermore, there is no selective loss of Lyt 1 bright cells after cytotoxic depletion of CRT (data not shown). (3) Complement-mediated, antibody-dependent cytotoxicity only detects surface antigen expression when the antigen is recognised by antibody capable of binding complement, for example, antibody of the IgG2 or IgM classes. Most of the presently available highly cytotoxic pools of anti-Lyt antisera contain considerable amounts of non-cytotoxic IgG1, anti-Lyt antibody (manuscript in preparation), which may block effective complement-mediated cytotoxicity. Using a selected pool of anti-Lyt 1.2, with no detectable IgG1, we have been able to kill >90% of CRT. (4) Other cell surface changes related to the differentiated state of the CRT may make cells more or less sensitive to antiserum plus complement. Recent work has suggested that specific lipids inserted into the membranes of tumour cells reduce the sensitivity to such cytotoxicity[20]. (5) The genetic locus for Lyt 1 may be a complex with more than one specificity. For example, a specificity, Lyt 1.1^a, may define a T^H subset capable of helper cell function, whereas Lyt 1.1^b defines a T-cell subset required for delayed-type hypersensitivity. These specificities could potentially be further defined by genetic differences, or the use of monoclonal antibodies obtained by anti-Lyt-myeloma fusions. Extensive analysis of the Lyt 1 alleles has been complicated by the fact that the titre of the anti-Lyt 1.2 antisera is much lower than that of anti-Lyt 1.1 antisera. This has also hindered comparative analysis of these antigens from previous cytotoxic data[19,21]. However, results with a limited number of anti-Lyt 1.2 antiserum pools indicate that expression of Lyt 1.2 closely resembles that of Lyt 1.1 in the FMF immunofluorescence analysis of thymocytes, CRT and peripheral T cells. Furthermore, preliminary evidence suggests that peripheral T cells display similar Lyt 123 phenotypes to those detailed here for CRT.

The accepted hypothesis suggests that all thymocytes and therefore all peripheral T cells express Lyt 123 during differentiation[3]. Our data suggest that there may be two lines of thymic differentiation; one line which expresses Lyt 1 but not Lyt 2 or 3 and a second line which expresses all three antigens during differentiation. Thus, there would be two thymocyte sublines corresponding to Lyt 1$^+$ cells and Lyt 123$^+$ cells. The functional capabilities of the peripheral T cells could be determined by or reflect the phenotype expressed in the thymus.

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Substance P causes direct depolarisation of neurones of guinea pig interpeduncular nucleus *in vitro*

SEVERAL lines of evidence suggest that the undecapeptide, substance P, is involved in synaptic transmission in various areas of the central nervous system[1]. The development of a specific radioimmunoassay and new immunohistochemical techniques has allowed the identification of several specific but putative pathways involving substance P in the brain[2,3]. In particular, the cell bodies in the medial habenula and probable nerve terminals in the interpeduncular nucleus contain high concentration of substance P[3]. In addition, lesioning of the habenula causes a drastic decrease in the substance P concentration in the interpeduncular nucleus[4]. These findings imply that substance P may act as a transmitter in the habenulo-interpeduncular system. Therefore, in the present study, the effect of substance P has been examined *in vitro* on the interpeduncular neurones in an isolated guinea pig brain stem preparation. Substance P depolarised the membrane of the interpeduncular neurones. This finding supports the possible transmitter role of substance P in the central nervous system.

The brain of an adult guinea pig of either sex was removed from the skull, placed on filter paper soaked with Krebs solution with the ventral surface of the brain facing upwards and divided along the midline. A block of the brain stem which included the thalamus, hypothalamus, mamillary body and interpeduncular nucleus was made by removing the majority of the brainstem, hippocampus and the cerebral cortex. A slice ~500–600 µm thick was cut from the medial surface using a stainless steel slicer. Incubation, stimulation and recording procedures are described in detail elsewhere[5]. The slice was continuously perfused with Krebs solution equilibrated with 97% O_2 and 3% CO_2 at 36 °C. Under a binocular microscope the stimulating electrodes, consisting of a pair of tungsten wires were placed gently on the habenulo-interpeduncular tract. For extracellular and intracellular single cell recordings, conventional glass microelectrodes were used.

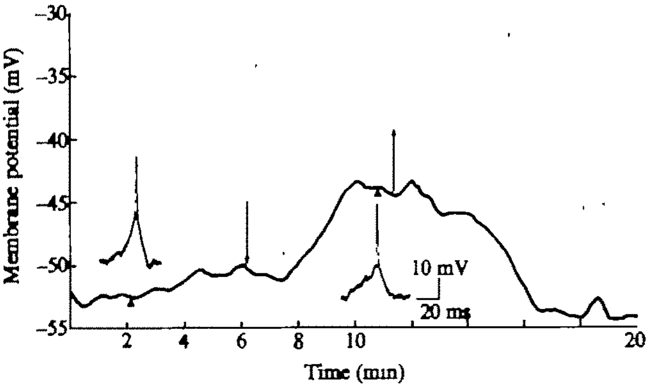

Fig. 1 Effects of substance P on the resting membrane potential of the interpeduncular neurone. Upward and downward arrows represent the duration of a perfusion with a Krebs solution containing 10^{-7} M substance P and the commencement of washing with normal Krebs solution. Inserts illustrate action potentials recorded at points indicated by (△) and (▲). Upward deflections represent positive polarity.

Intracellular recordings from interpeduncular neurones show their resting membrane potential to be 52.8±6.7 mV. Each neurone responded to stimulation of the habenulo-interpeduncular tract (50–100 μs duration) with an excitatory postsynaptic potential which evoked an action potential. No hyperpolarising potentials were observed. In most records spontaneous firing occurred.

In 10 stable extracellular records, substance P (10^{-7} M) was shown to increase reversibly the firing rate of the interpeduncular neurones 1.5 to 5-fold except for one neurone on which no obvious change occurred. L-glutamate also increased the firing rate of the interpeduncular neurones and dose–response curves show 10^{-5} M L-glutamate to be roughly equipotent with 10^{-7} M substance P. In Fig. 1, intracellular records show that substance P (10^{-7} M) induced a depolarisation of ~8 mV. The original membrane potential was restored by washing. The excitatory action of substance P was preserved in low calcium medium.

Although the excitatory action of substance P observed in the present study may be nonspecific, comparable studies on slices cut from the hippocampus and cerebellar cortex which contain almost no substance P show substance P to exert no effect. Similar negative results were reported by Dodd and Kelly[6].

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Depolarisation-induced changes in muscarinic cholinergic receptors in synaptosomes

PROLONGED exposure of receptors to ligands can result in a reduction in the number of available binding sites[1–8]. This constitutes one possible homeostatic mechanism in the control of interneural communication as there is a functional desensitisation of target tissues. The association between transmission-linked events and the extent of ligand binding can be investigated conveniently in synaptosome preparations. Here we present evidence for what may be a similar desensitisation phenomenon for pre- or postsynaptic muscarinic acetylcholine receptors on synaptosomal membranes as a result of depolarisation mediated via prolonged or persistent opening of sodium channels.

Depolarisation of synaptosomes and cells by electrical stimulation or veratrine treatment cause, through membrane depolarisation, augmentation of metabolic rates, Ca^{2+} uptake, membrane protein phosphorylation, Ca^{2+} dependent transmitter release, cyclic GMP levels, and increased turnover of neurotransmitters[9–13]. All these effects, together with K^+ efflux, are abolished or greatly reduced by blocking the Na^+ channels with tetrodotoxin, which, in the absence of stimulation, has no observable effect[9,10,12,13].

Muscarinic receptors in the rat brain have been characterised in detail using tritiated agonists and antagonists[14–18]. The binding of one such radiolabelled antagonist, [^{3}H]*N*-methylatropine (^{3}H-NMA), to muscarinic receptors on synaptosomal membranes was greatly reduced (up to 50%) following treatment of synaptosome preparations[19] with electrical pulses, and to a lesser extent, after veratrine treatment. This effect was totally abolished when tetrodotoxin (1 μM) was present during stimulation (Table 1). With increasing duration of stimulation, the number of receptors decreased at a considerably faster rate than did the control unstimulated, synaptosome membranes (Fig. 1*a*). In addition, the extent of the reduction in ligand binding appeared to be proportional to stimulus size over the current range 600–800 mA (Fig. 1*b*). Depolarisation with 56 mM K^+ in contrast produced no change in the number of antagonist binding sites.

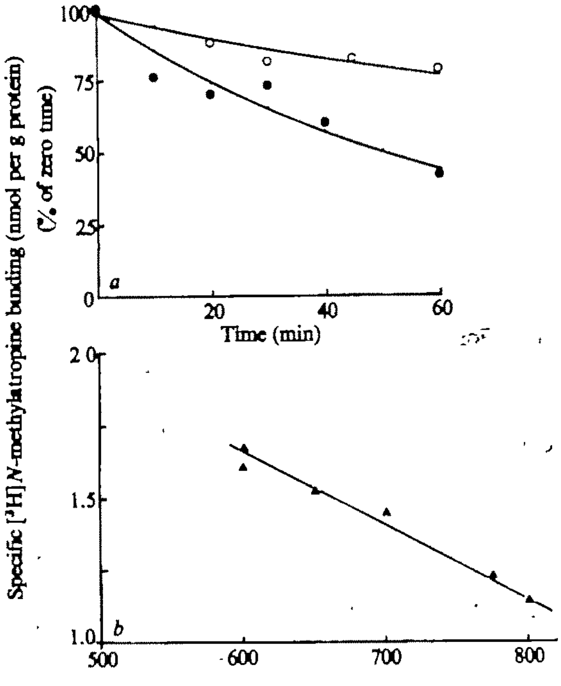

Fig. 1 Time and stimulus-dependence of synaptosomal receptor population. Synaptosomes were prepared from cerebral cortex[19] and incubated at 37 °C in Krebs-phosphate medium containing 10 mM glucose in Warburg respirometer flasks. *a*, Stimulation was applied through gold-ring electrodes for the times indicated, and the reaction was terminated by centrifugation. Hypo-osmotic lysis of the resultant pellets, at 0 °C in 10 mM phosphate buffer *p*H 7.0 followed by centrifugation of the lysed suspension gave a fraction of synaptosomal membranes. These were stored at −80 °C before measurement of receptor binding capacity as described in Table 1 legend. The open symbols and closed symbols represent binding capacities in the absence and presence respectively of electrical stimulation. *b*, The relationship between receptor loss and the size of the stimulus was investigated by changing the current delivered through the electrodes. In each case, stimulation lasted for 30 min. A typical experiment is shown for a group of six samples each of which showed a different rate of decline of antagonist binding to the muscarinic receptors.

It may be that the depolarisation-induced loss of ^{3}H-antagonist binding is due to the action of cytoplasmic or lysosomal enzymes (or other soluble factors) released from synaptosomes. These could modify the ligand-binding properties of receptors by proteolysis or other biochemical actions. This possibility was investigated in several ways. The presence of chymotrypsin (3 μM) during the 1-h synaptosome incubation did not cause any reduction in binding nor did inclusion of phenylmethyl sulphonyl fluoride (a serine protease inhibitor) at 30 μM prevent the electrically stimulated loss. The effects of endogenous synaptosomal proteolytic enzymes on muscarinic receptors were tested by adding synaptosomal lysates to intact synaptosome preparations. Addition of freeze-dried protein

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Table 1 Effect of depolarising agents and neurotransmitters on subsequent muscarinic antagonist binding to synaptosomal membranes

	^{3}H-antagonist bound per g synaptosomal protein (% of control values)									
	Electrical pulses		Veratrine		K$^+$		Glutamate		Carbachol	
Control	100	(8)	100	(9)	100	(6)	100	(4)	100	(8)
Tetrodotoxin	95±2	(3)	98	(2)	—		—		—	
Stimulation	58±5*	(15)	83±2	(9)*	106±4	(9)	107±5	(6)	105±4	(17)
Stimulation in presence of tetrodotoxin	93±1	(4)	97±4	(5)	—		—		—	

Synaptosomes prepared from rat cerebral cortex[19] were incubated at 37 °C in Krebs-phosphate medium containing 10 mM glucose and tetrodotoxin (1 μM), where appropriate, in Warburg respirometer flasks. After preincubation for 30 min stimulation was applied by addition of veratrine (76 μM), potassium (56 mM), glutamate (100 and 200 μM) or carbachol (0.1–100 μM) or by application of electrical pulses[12] for 30 min. Pelleted synaptosomes were then exposed to hypo-osmotic lysis at 0 °C in 10 mM phosphate buffer pH 7.0 and the pellet of synaptosomal membranes obtained after centrifugation (100,000g, 10 min) stored at −80 °C. Subsequent receptor binding was performed as described previously[16] using saturating concentrations of [^{3}H] N-methylatropine (10^{-8} M) and a rapid centrifugation method[17]. Nonspecific binding, determined by incubating the membranes and ^{3}H-NMA with a high concentration (1 μM) of 3-quinuclidinylbenzilate or atropine, was in general less than 10% of the specific binding. Values for controls were in the range 1–2 nmol ^{3}H-antagonist per g synaptosomal protein. Results are means ± s.e.m. for the number of independent experiments given in brackets.

* Significantly different from control, $P < 0.005$ (using a completely randomised analysis of variance).

80 μg ml^{-1} (equivalent to 20% of the total osmotically releasable synaptosomal protein) to the incubation did not reduce the binding of ^{3}H-NMA to the receptors. A concentration of 150 μg ml^{-1} was required to produce a 15–20% loss in binding over 1 h. The effects shown in Table 1 would require lysis of 50–80% of the incubated synaptosomes on electrical stimulation; such damage has not been observed in previous experiments[11,12]. As the 'proteolytic potency' of the lysate may have been reduced by extraction and freeze-drying, synaptosomal membranes were incubated in the medium obtained from a previous incubation of intact stimulated synaptosomes. This medium would contain any released soluble enzymes. No loss of binding of ^{3}H-NMA was observed in this experiment. Furthermore, the loss in numbers of muscarinic receptors was not accompanied by a change in activity of the membrane-bound enzymes, Na$^+$/K$^+$-ATPase or acetylcholinesterase, or any detectable changes in the Coomassie blue staining pattern of the protein bands of solubilised synaptosomal membranes, when electrophoresed on SDS-polyacrylamide gels. In these experiments, electrical stimulation of synaptosomes prelabelled with the irreversible label, ^{3}H-propylbenzilylcholine mustard[15], showed no evidence of proteolysis of the specifically labelled 83,000 molecular weight[20] binding site of the muscarinic receptor or of release of the membrane-bound receptor into the medium. Thus the observed fall in the antagonist binding capacity seems to be a specific effect linked to the depolarisation event or its sequelae rather than to any gross degenerative effects triggered by the stimulus.

The exposure of crude synaptosome preparations to high concentrations of muscarinic agonists is apparently without effect on the subsequent time-dependent binding kinetics of agonists or antagonists to the receptor population[16,18]. Our results (Table 1) similarly showed that pretreatment of synaptosomes with carbachol, or glutamate (to simulate transmitter release) or potassium (which is released during depolarisation) had no effect on subsequent ^{3}H-NMA binding.

Our experiments suggest that the decreased binding of ^{3}H-NMA to muscarinic receptors on electrical stimulation or on veratrine treatment is not a direct effect of these agents since the receptor 'loss' is inhibited by low concentrations of tetrodotoxin, which blocks sodium channels. The decreased binding seems to be a result of depolarisation of the synaptosome. One of the main consequences of depolarisation is the release of neurotransmitters. However, the loss of binding activity does not seem to be a result of neurotransmitter–receptor interactions as the phenomenon is not triggered by treatment of the synaptosomes with carbachol or glutamate. Hence the phenomenon is different from the reported agonist-induced loss of β-adrenergic receptors[6,7]. Furthermore, the fact that K$^+$ depolarisation does not decrease ^{3}H-NMA binding suggests that the receptor loss is specifically associated with the persistent opening of Na$^+$ channels.

One possibility is that an agent, released with the neurotransmitter (presumably acetylcholine) modifies the muscarinic receptor. However, any modification of the receptor does not seem to be a general proteolysis; the molecular weight of the receptor and the activity of certain membrane-bound enzymes are not altered by electrical stimulation. The hypothetical factor would have only a transient activity in reducing binding to the receptors in view of the fact that incubation of the medium from electrically stimulated synaptosomes with intact synaptosomes has no effect on ^{3}H-NMA binding. As synaptosomes consist of intact presynaptic structures, commonly carrying parts of the postsynaptic membranes and thickenings, such a factor would be released, along with the neurotransmitter, in high concentration into the synaptic cleft. Any such factor could therefore rapidly modify both postsynaptic and presynaptic receptors before inactivation or diffusion from the cleft.

An alternative possibility that the loss is principally of presynaptic receptors located on the synaptosome membrane and is caused by endocytosis of receptors or a modification of this membrane which is initiated by depolarisation. This hypothesis would imply that at least 50% of the receptors in the preparation were localised presynaptically as this is the observed loss of ^{3}H-NMA binding. There is evidence for the presynaptic localisation of muscarinic receptors[21,22]: in the present experiments carbachol (200 μM) stimulated amino acid release by 30–80%.

One can speculate that the modification of the binding properties of the muscarinic receptor, if reversible *in vivo*, could provide a mechanism for desensitisation, which is known to occur with considerable facility in muscarinic receptor-linked responses[23,24]. The mechanism of receptor 'loss' is being investigated further.

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Voltage-dependent calcium current induced by serotonin

CALCIUM ions have a central role in the regulation of neural activity[1,2]. Changes in intracellular calcium concentration affect membrane permeability to other ions and thereby alter membrane excitability[3]. Furthermore, calcium is required for transmitter release from nerve terminals[4]. A neurotransmitter that specifically induces an inward calcium current might have profound effects. We report here that in some neurones of *Aplysia*, serotonin can induce such a calcium current. A previous investigation[5] revealed that iontophoretically applied serotonin elicited an unusual excitatory response in some neurones of *Aplysia*. At hyperpolarised potentials the neurotransmitter produced a slow excitation (component 1) that behaved simply as an increase in sodium conductance, similar to the A′ response of Gerschenfeld and Paupardin–Tritsch[6]. At depolarised potentials (>−30 mV), the response was voltage dependent and behaved as a regenerative inward current (component 2). At all potentials, the slow excitatory response to serotonin was insensitive to changes in extracellular chloride or potassium concentration. However, in sucrose-substituted sodium-free solutions, the response was greatly attenuated. Although a calcium current was not excluded, it was tentatively suggested that sodium was responsible for the regenerative inward current as well as for component 1. Further study has now shown that the regenerative inward current component is carried by calcium ions.

The slow excitatory response was elicited by iontophoretic application of serotonin on unidentified neurones of the left caudal quarter of the abdominal ganglion and cells B1 and B2 of the buccal ganglia of *Aplysia californica*. Iontophoretic pulses of serotonin were applied at a constant rate (once every 5 min) throughout the experiment. Neurones were voltage clamped by the single-electrode method of Wilson and Goldner[7]. Methods are the same as those described previously[5].

Calcium currents in many preparations are blocked by the divalent cations cobalt[1,8,9] and manganese[1,9,10]. Figure 1*a* shows that component 2 of the excitatory response to serotonin is strongly attenuated by 25 mM cobalt in calcium-free seawater. This was consistently observed in all of the seven cells tested with cobalt. Component 1 was affected to varying degrees; in 3 cells it was minimally affected, but in four it was greatly reduced. In the cell illustrated, component 1 was only slightly attenuated. Manganese (25 mM in calcium-free seawater) was also a potent blocker of the excitatory response to serotonin (Fig. 1*b*). In the six cells tested, it reversibly attenuated both components of the excitatory response to serotonin. The actions of manganese on component 1 are reminiscent of the effects of this divalent cation on the fast sodium-dependent response to dopamine observed by Ascher[11].

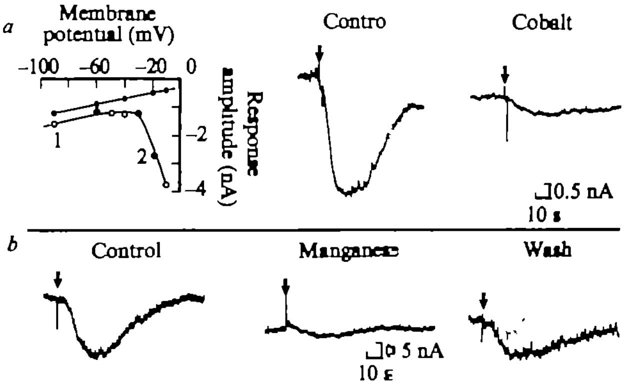

Fig. 1 Actions of calcium blockers cobalt and manganese. The graph in *a* shows that 25 mM cobalt in calcium-free seawater blocks component 2 (the inward current at potentials more depolarised than −30 mV) but minimally affects component 1 (the inward current at more hyperpolarised potentials). The current traces in *a* are from the same cell and show responses to serotonin when the membrane potential was voltage clamped to −10 mV in normal seawater (Control) and in the presence of cobalt. Inward current is represented by a downward deflection. *b* Shows the current response to serotonin in another cell when the membrane potential was clamped to −14 mV while in normal seawater (Control), in 25 mM manganese in normal seawater, and following replacement of normal seawater (Wash) ○, Control; ●, 25 mM CO^{2+}.

When extracellular sodium was replaced with glucosamine, component 1 was attenuated within 15 min. This effect was rapidly reversed with the reintroduction of sodium. As shown in Fig. 2, component 2 was affected differently, in that the response to serotonin was prolonged. In two of the three cells studied, the response was still present after 40–50 min in sodium-free seawater. In the third cell, component 2 of the response was initially prolonged, but within 40 min had declined to zero amplitude. The response recovered slowly in normal seawater, requiring 60 min for 50% reversal. Thus, component 1 is always quickly and reversibly blocked by sodium-free solutions, whereas component 2 is either left unblocked or is very slowly attenuated. In squid axon, prolonged exposure to sodium-free solutions increases intracellular free calcium[12], and intracellular accumulation of calcium has been shown to block an inward calcium current[13,14]. This may be the basis of our observed depression of component 2 in sodium-free solutions. It was previously reported that the serotonin response was greatly reduced by sucrose-substituted sodium-free seawater[5]. The present results using other Na^+ substitutes suggest that this effect may be related to the decreased ionic strength of the solution or to some nonspecific action, rather than the reduced sodium concentration.

The different ionic sensitivities of the two components of the excitatory response to serotonin indicate that these are independent conductance changes. This is further supported by the observation that in some cells only component 1 was observed, whereas in others only component 2 could be elicited by iontophoresis of serotonin. Component 1 is probably the A′ response of Gerschenfeld and Paupardin-Tritsch[6] and is due to an increase in sodium conductance. The results presented here suggest that calcium is carrying the regenerative inward current at depolarised potentials (component 2).

This is the first demonstration that iontophoretic application of a neurotransmitter can elicit a current carried exclusively by calcium. Of further interest is the regenerative nature of the inward current. Regenerative inward calcium currents have been observed in other systems. A regenerative calcium current is responsible for the calcium component of action potentials[1]. It is also thought to underlie the burst firing behaviour of neurones[15,16], and Eckert and Lux[15] have suggested that, in

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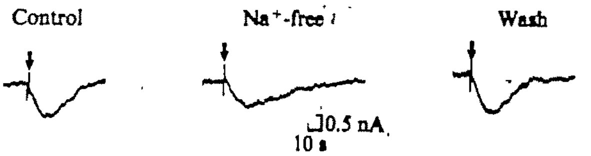

Fig. 2 Effects of sodium-free seawater on current response to serotonin. Na$^+$-free trace was taken after 55 min in sodium-free solution. Membrane potential was voltage clamped to −18 mV. Glucosamine HCl completely replaced NaCl in the seawater. The pH of the solution was adjusted to 7.2 by addition of 60 mM $Mg(OH)_2$. 'Control' and 'Wash' solutions were normal seawater at pH 7.2 to which 60 mM $MgCl_2$ had been added.

Helix, this current is carried exclusively by calcium. At the presynaptic terminal of squid giant synapse, Katz and Miledi[10] characterised a regenerative inward calcium current that they hypothesised was responsible for transmitter release. Induction of a regenerative calcium current by serotonin may serve to modulate one or more of these processes.

Demonstration of a neurotransmitter-evoked calcium current suggests the possibility of transmitter regulation of intracellular calcium concentration. As intracellular calcium is a principal regulator of a variety of cell functions, this transmitter action is of great physiological significance. Modulation of transmitter release is one possible role for a serotonin-induced calcium current. Our finding may explain some observations reported in the literature. Shimahara and Tauc[17] and Kandel *et al.*[18] have proposed that serotonin mediates presynaptic facilitation. In some cells of *Aplysia*, serotonin can broaden action potentials in the presence of tetraethylammonium, and Klein and Kandel[19] suggested that a calcium current is modulated by the neurotransmitter. Dretchen *et al.*[20] have observed that transmitter release at the mammalian neuromuscular junction is enhanced by serotonin, perhaps through a cyclic AMP mechanism. In neuroblastoma–glioma hybrid cells cultured with normal mouse myotubes, Christian *et al.*[21] have shown that serotonin caused release of acetylcholine and facilitated synaptic release. The regenerative inward calcium current induced by serotonin described here may underlie the presynaptic modulatory actions of serotonin, and may also have other important, as yet unrecognised, regulatory effects on neuronal function.

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Inhibition of Ca^{2+} efflux from mitochondria by nupercaine and tetracaine

IT was reported by Mela[1,2] that local anaesthetics stimulate Ca^{2+} uptake by rat liver mitochondria. This has been attributed to the protective action of these compounds on mitochondrial structure[3,4], particularly with regard to inhibition of phospholipase A (refs 5, 6). However, another possibility is that local anaesthetics affect differentially, and more directly, the uptake and efflux of Ca^{2+}. Recent evidence suggests that mammalian mitochondria, in addition to the well documented route for Ca^{2+} accumulation, have a different mechanism for Ca^{2+} release[7–9]. In the case of rat liver mitochondria, inhibition of Ca^{2+} uptake by the addition of

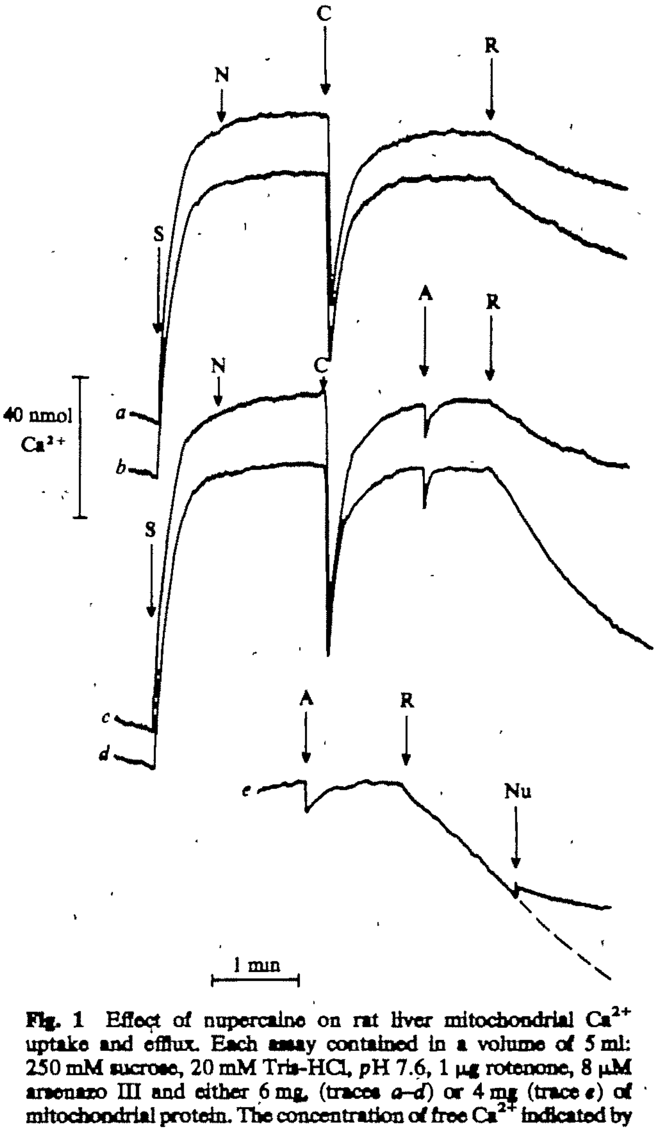

Fig. 1 Effect of nupercaine on rat liver mitochondrial Ca^{2+} uptake and efflux. Each assay contained in a volume of 5 ml: 250 mM sucrose, 20 mM Tris-HCl, pH 7.6, 1 μg rotenone, 8 μM arsenazo III and either 6 mg (traces *a–d*) or 4 mg (trace *e*) of mitochondrial protein. The concentration of free Ca^{2+} indicated by arsenazo III was measured using the absorbance difference 685–665 nm. A downward deflection of the trace indicates increasing extra-mitochondrial Ca^{2+}. The temperature was 30 °C. For traces *a–d*, succinate (12.5 μmol) was added at S, calcium chloride (50 nmol) at C and ruthenium red (6 nmol) at R. For traces *a* and *c*, 0.3 μmol nupercaine was added at N and for traces *c* and *d*, 12.5 μmol acetoacetic acid (Li salt) was added at A. For trace *e*, the experimental technique was exactly as for trace *d* up to the addition of 12.5 μmol acetoacetate at A. 4 nmol ruthenium red was added at R and 0.5 μmol nupercaine at Nu. The initial part of the trace is omitted for clarity. The dotted line represents the course of Ca^{2+} efflux in the absence of nupercaine.

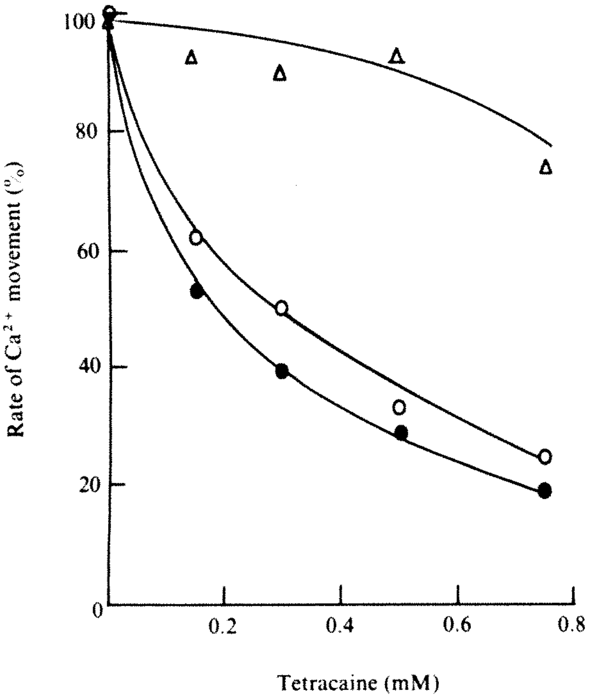

Fig. 2 Effects of tetracaine on Ca^{2+} uptake and efflux. The experimental procedure was the same as for Fig. 1, traces *a–d*, except that for measurement of the rate of Ca^{2+} uptake, the time axis was expanded 2.5×. Each assay contained 8 mg rat liver mitochondrial protein. △, Initial rate of Ca^{2+} uptake after the addition of 50 nmol Ca^{2+}; ○, ●, initial rate of efflux after addition of ruthenium red. In ●, 12.5 μmol acetoacetate was added 1 min before ruthenium red.

ruthenium red causes a slow release of Ca^{2+} from the mitochondrial matrix[7,8] which is enhanced by substrates such as acetoacetate, which cause oxidation of intra-mitochondrial NADH (ref. 10). In mitochondria from other tissues, such as heart and brain[8,9], the ruthenium red-insensitive release is dependent on Na^+ ions. We report here, using mitochondria from both rat liver and brain, that nupercaine and tetracaine, at concentrations which do not inhibit Ca^{2+} uptake, cause considerable inhibition of the ruthenium red-insensitive Ca^{2+} efflux observed in energised conditions.

Rat liver mitochondria were prepared as previously described[11], except that 0.5 mM EGTA was included in the homogenisation and first-wash media. Non-synaptosomal rat brain mitochondria were isolated by the method of Garbus[12] in a medium containing 320 mM sucrose, 5 mM HEPES–KOH, *p*H 7.6, and 0.5 mM EDTA, followed by purification on a discontinuous Ficoll gradient as described by Nicholls[9]. Ca^{2+} uptake and output were measured using arsenazo III as an extra-mitochondrial Ca^{2+} indicator[13] in an Aminco-Chance dual wavelength spectrophotometer equipped with a stirred cuvette. The overall response time was approximately 0.5 s.

Figure 1*b* shows the standard procedure used for our experiments with rat liver mitochondria. The addition of succinate causes the rapid uptake of free Ca^{2+} from the medium (40 nmol) plus Ca^{2+} which has leaked out of the mitochondria before the initiation of respiration. After 2 min the mitochondria were loaded with a further 50 nmol Ca^{2+} (as $CaCl_2$). Then, after a further 2 min, uptake was inhibited by the addition of 1 nmol ruthenium red per mg protein. This resulted in a slow Ca^{2+} efflux, as described previously[7,8]. In Fig. 1*a*, 60 μM nupercaine was added before Ca^{2+}. It can be seen that this has no effect on the kinetics of Ca^{2+} loading, but the subsequent efflux rate after ruthenium red addition is inhibited to approximately 50% of the control rate. When 2.5 mM acetoacetate is present for 1 min before ruthenium red, the initial rate of efflux is approximately doubled (Fig. 1*b*, *d*). Figure 1*c* shows that this higher rate is also 50% inhibited by 60 μM nupercaine.

Although in these experiments the anaesthetic was in contact with the mitochondria for $3\frac{1}{4}$ min, preincubation is not necessary. Figure 1*e* shows that the addition of 100 μM nupercaine after efflux has been initiated causes an essentially immediate inhibition of Ca^{2+} output. This result seems to rule out the possibility that effects due to preservation of mitochondrial integrity are involved in the inhibition of efflux, as these would presumably require the presence of the anaesthetic throughout the experiment.

Figure 2 summarises data for tetracaine, including its effect on the velocity of Ca^{2+} uptake. It can be seen that concentrations of the anaesthetic which cause up to 70% inhibition of efflux have essentially no effect on uptake, although at higher concentrations uptake is impaired to some extent. Preliminary observations with butacaine suggest that it behaves similarly to tetracaine, but there is a less clear distinction between its effects on uptake and efflux. The concentration of butacaine required for 50% inhibition of efflux is ~0.8 mM, compared with 0.2 mM for tetracaine and 0.06 mM for nupercaine.

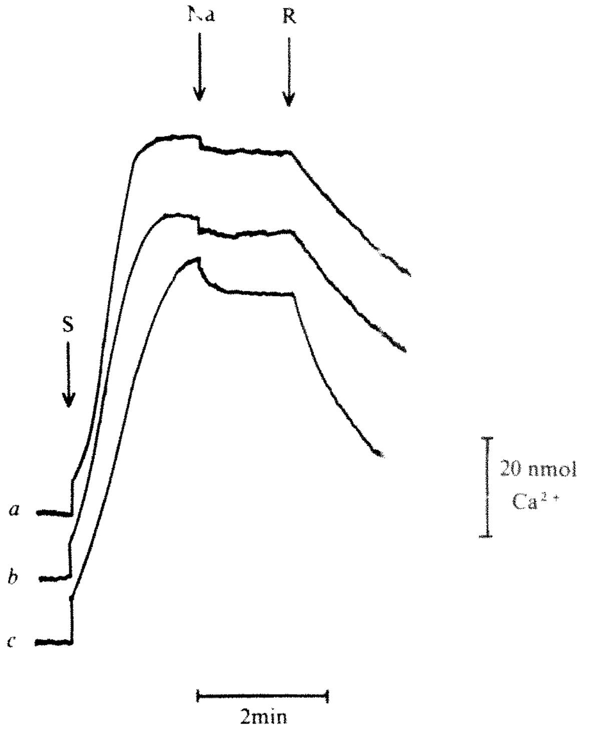

Fig. 3 Effects of nupercaine and tetracaine on rat brain mitochondrial Ca^{2+} uptake and efflux. Each assay contained in 5 ml: 250 mM sucrose, 20 mM Tris-HCl, *p*H 7.6, 1 μg rotenone, 1 mM potassium phosphate, *p*H 7.6, 6 μM arsenazo III, 12 μM free Ca^{2+} (measured with a Ca^{2+}-sensitive electrode) and 2.4 mg mitchondrial protein. For all traces: S indicates the addition of 12.5 μmol succinate (K^+ salt); Na, 25 μmol NaCl; R, 3 nmol ruthenium red. In addition, experiment *a* contained 1.1 μmol nupercaine and *b* 2.5 μmol tetracaine, both added 30 s before succinate. Other conditions were as for Fig. 1.

Figure 3 shows the effects of nupercaine and tetracaine on Ca^{2+} transport by rat brain mitochondria. In the conditions of this experiment, both compounds produce a considerable stimulation of uptake, an almost complete inhibition of the Na^+-induced efflux and a 50% inhibition of the rate of Ca^{2+} release subsequent to ruthenium red addition. The nature of the stimulation of uptake is not clear, but one possibility is a decrease in Ca^{2+} cycling during uptake.

Because the local anaesthetics used in this study all carry a positive charge, it might be expected that when they bind to a membrane they would reduce its passive permeability to cations[14]. Also, local anaesthetics have been shown to compete with Ca^{2+} for binding sites in the mitochondrial membrane[15]. It is rather surprising, therefore, that, at low concentrations, nupercaine and tetracaine inhibit Ca^{2+} efflux and have little effect on, or stimulate, Ca^{2+} uptake. Also, we have found that the rapid efflux of Ca^{2+} brought about by collapse of the membrane potential by uncouplers or antimycin A (ref. 7) is not affected by tetracaine, either in the presence or absence of ruthenium red. The inhibition of efflux in energised conditions thus seems to be a relatively specific effect, and provides further evidence for the view that the route for Ca^{2+} efflux from mitochondria is different from that for uptake. It is clearly a possibility that if mitochondria are involved in the regulation of cytosolic Ca^{2+} concentration, then this concentration might be perturbed by the application of nupercaine or tetracaine. Such a perturbation of external Ca^{2+} concentration would not be readily observed in the experimental conditions shown in Fig. 1, as even in the absence of nupercaine, measurements with a Ca^{2+}-sensitive electrode show that the mitochondria reduce the free external Ca^{2+} concentration to approximately 1 μM. If nupercaine stimulated the uptake of some, or even all, of this residual Ca^{2+}, the resulting change in absorbance of arsenazo III would be very small. It has, however, been observed[16] that the application of 0.1–1 mM tetracaine to perfused liver blocks both glucagon and cyclic AMP stimulated gluconeogenesis and Ca^{2+} release into the perfusate.

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Gene fusion during the evolution of the tryptophan operon in Enterobacteriaceae

THE genes of tryptophan biosynthesis are organised differently in various species of microorganisms[1]. In several organisms it seems that gene fusions have joined functionally distinct polypeptide chains into single bifunctional proteins. Here we present our analysis of one example of a possible gene fusion and an attempt to deduce the sequence of events responsible.

The two initial reactions specific to the tryptophan pathway

$$\text{chorismate} + \text{L-glutamine} \xrightarrow{Mg^{2+}} \text{anthranilate} + \text{pyruvate} + \text{glutamate} \quad (1)$$

$$\text{anthranilate} + \text{5-phosphoribosylpyrophosphate} \xrightarrow{Mg^{2+}} N\text{-(5'-phosphoribosyl)anthranilate} + \text{pyrophosphate} \quad (2)$$

are catalysed by the enzymes anthranilate synthetase (AS) and anthranilate-5-phosphoribosylpyrophosphate phosphoribosyl transferase (PRT) respectively. In many bacterial genera[1,2], AS is an enzyme complex containing two non-identical polypeptides, components I and II[3]. Component I alone can convert chorismate to anthranilate using NH_3 but not L-glutamine as the amino donor. Component II contributes glutamine amido transferase (GAT) activity to the complex, thus conferring the ability to use L-glutamine as the amino donor[2]. While the size of AS component I is fairly uniform in bacteria[2], at least two different forms of component II have been observed. In *Escherichia coli*[4], and *Salmonella typhimurium*[5] it has a molecular weight of about 65,000 and has two distinct enzymatic capabilities: it provides the GAT activity for reaction (1) when complexed with component I, and catalyses reaction (2), the PRT reaction, whether in the complex or not. In *Serratia marcescens* a small polypeptide with a molecular weight of 21,000 contributes the GAT activity to AS[6–8], but a separate polypeptide with a molecular weight of 45,000[8,9] has PRT activity.

The two enzyme activities of AS component II of *E. coli* and *S. typhimurium* are associated with different segments of the polypeptide chain. Genetic studies indicate that the GAT activity resides in the N-terminal third of the molecule while PRT activity is associated with the remainder of the polypeptide chain[10–12]. Mild proteolytic digestion of intact component I–component II complexes of *E. coli* or *S. typhimurium*[8,13] produces a component II fragment of molecular weight 23,000 which retains GAT activity but lacks PRT activity. Li *et al.*[8] have found extensive amino acid sequence homology at the N-terminal end of these component II fragments of *E. coli* and *S. typhimurium* and the functionally homologous intact component II GAT polypeptide of *S. marcescens.* This suggests that the GAT region of the bifunctional component II polypeptides of *E. coli* and *S. typhimurium* and the smaller GAT subunit of *S. marcescens* share the same evolutionary origin and lends direct support to the idea that the bifunctional component II polypeptides of *E. coli* and *S. typhimurium* arose by fusion of two separate genes, one coding for a GAT and a second coding for PRT[5,8,11,14].

In *E. coli* all the genes of tryptophan biosynthesis are organised as an operon with the structural gene for AS component I (*trpE*) closest to the promoter/operator region[15]. *trpE* is followed by the structural gene for AS component II (*trpD*), with the genetic region specifying the GAT portion of the polypeptide (*trpG*) closest to *trpE*[8]. In order to indicate the two domains of the structural gene for the bifunctional component II polypeptide of *E. coli,* we shall designate it *trp*(*G*)*D* (ref. 1). In *S. marcescens*, the evidence for the organisation of the genes of tryptophan biosynthesis as an operon is indirect[9]. In particular, it was uncertain whether the structural gene for the GAT subunit of AS, *trpG*, lies adjacent to *trpD*, the PRT structural gene, or whether it is unlinked to the remaining genes of the operon, as in *Bacillus subtilis*, where AS component II also seems to provide GAT function for *p*-aminobenzoate synthesis[16,17].

We have recently characterised a cloned DNA fragment containing the regulatory region and first structural gene, *trpE*, of the *S. marcescens trp* operon[18]. DNA sequence analysis revealed that the fragment also contains the sequence coding for the first nine amino acids at the N-terminus of AS component II, confirming that *trpG* lies adjacent to *trpE* in the *trp* operon of *S. marcescens*[18]. Here we report the cloning of a different DNA fragment containing all the structural genes of the *S. marcescens trp* operon with the exception of *trpA*. A functional and structural analysis of this DNA fragment established that the gene order at the promoter proximal end of the *S. marcescens trp* operon (*trpE–trpG–trpD*) is analogous to the arrangement in *E. coli.* We have determined the DNA nucleotide sequence of the *trpG–trpD* punctuation region in *S. marcescens* and of the analogous region of the fused *trp*(*G*)*D* gene of *E. coli.* A comparison of the two sequences reveals extensive homology

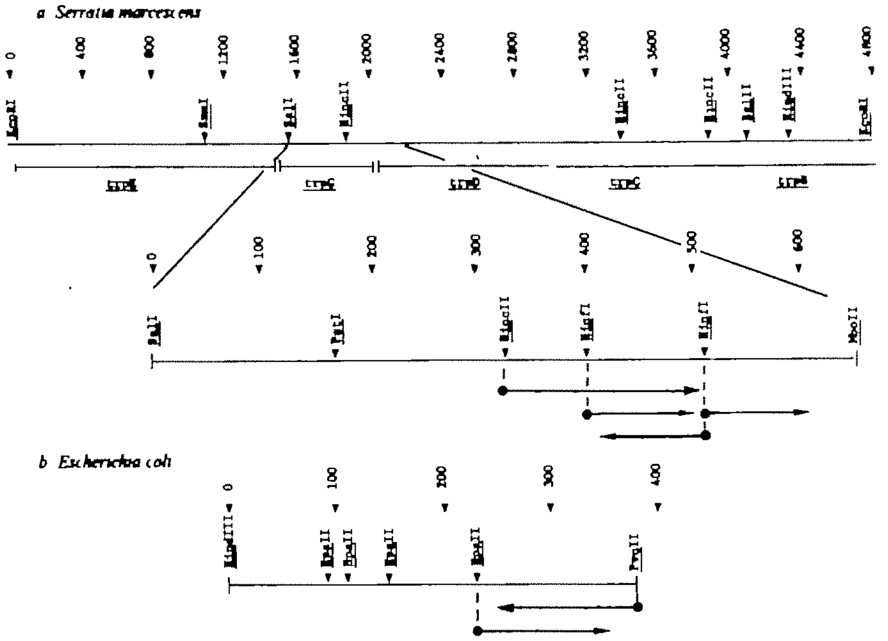

Fig. 1 *a*, Restriction map of a cloned fragment containing *S. marcescens trpEDCB*. Chromosomal DNA of *S. marcescens* was prepared by the method of Saito and Miura[37]. 10 μg of chromosomal DNA and 5 μg of pBR322 DNA were digested with *Eco*RI, phenol extracted, ethanol precipitated and resuspended in 250 μl of ligation buffer (60 mM Tris-HCl, *p*H 7.6, 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP). The mixture was incubated in the presence of T4 DNA ligase for 2 h at 13 °C. Ligation was stopped by ethanol precipitation and the pellet resuspended in 100 μl TE-buffer (10 mM Tris-HCl, *p*H 7.9, 0.1 mM EDTA). 20 μl were used to transform *E. coli* strain JA 221(C600 $r^- m^+ \Delta trpE5$ *leu recA*, obtained from J. Carbon) to prototrophy. Trp^+ transformants were screened for plasmid content. Plasmid pGM6 was recovered from such a transformant. It carries a single 4,800 base pair *Eco*RI insert. A restriction map of this insert is shown in the top portion of (*a*). The direction of the transcription is from left to right. The approximate location of the various *trp* genes is indicated underneath; the gene order in the dotted portion of the line has not been determined with certainty. The bottom portion of (*a*) shows an expanded restriction map of the DNA region containing the *trpG–trpD* interconnecting/intercistronic region. The arrows indicate the direction and length of sequencing runs, *b*, Restriction map of a DNA fragment from *E. coli* containing the *trpG–trpD* interconnecting region. *E. coli trpPOLED* DNA was isolated from plasmid pVH153[20]. The arrows underneath the restriction map indicate the length and direction of sequencing runs.

within the *trpG* and *trpD* structural genes, but considerable divergence in the *trpG–trpD* junction region. How the two sequences may be evolutionary related will be considered.

Chromosomal DNA of *Serratia marcescens*, obtained from wild-type strain SM6, was restricted with endonuclease *Eco*RI and the cleavage products were inserted into the *Eco*RI site of plasmid pBR322[19]. Plasmids carrying the initial portion of the *S. marcescens trp* operon were selected from the ligation mixture by their ability to transform an *E. coli* recipient strain which carries a deletion of most of *trpE* to tryptophan independence (see legend to Fig. 1 for experimental details). Plasmids pGM6, isolated from such a transformant, carries a single *Eco*RI insert of approximately 5,000 base pairs, whose restriction map appears in the top portion of Fig. 1*a*. pGM6 yields Trp^+ transformants with *trpE*, *trpD*, *trpC* and *trpB* but not *trpA* mutant recipients. Thus pGM6 carries most of the *S. marcescens trp* operon. The physical location and orientation of the *S. marcescens trp* genes on the *Eco*RI insert was determined by subcloning portions of the insert using various restriction enzymes. One such plasmid, pGM63, carries the 1,500-base pair *Eco*RI–*Sal*I segment from the left hand end of the *Eco*RI fragment shown in Fig. 1(ref. 18). This DNA fragment contains the entire *trpE* gene from *S. marcescens*[18]. The *Eco*RI site precedes the start of *trpE* by 20 base pairs, while the *Sal*I site is located approximately 30 base pairs beyond the *trpE–trpG* boundary[18]. Neither pGM6 nor pGM63 contains the promoter/operator region of the *S. marcescens trp* operon. Therefore, in both plasmids transcription of the *trp* genes must originate at a pBR322 promoter. The orientation of the insert is the same in both plasmids. Transcription is towards the region determining tetracycline resistance and away from the region determining ampicillin resistance[19].

We estimated the location of the *trpG–trpD* boundary of *S. marcescens* on the basis of the molecular weight of 21,000 for the *trpG* gene product[4,5]. The end of *trpG* and the presumed *trpG–trpD* intercistronic region was predicted to be approximately 500 base pairs from the *Sal*I site in the initial portion of *trpG*. A detailed restriction map of this DNA segment is shown in the bottom portion of Fig. 1*a*. DNA sequence analysis was performed with DNA fragments labelled at either the *Hin*fI sites (positions 400 and 520) or the *Hin*cII site (position 330) as outlined in Fig. 1*a*.

The analogous region of the *E. coli trp* operon is contained on plasmid pVH153[20]. This plasmid carries *E. coli trpPOLE*(*G*)*D* on a 4.7 megadalton *Eco*RI fragment from $\lambda trpED_{10}$ inserted into the *Eco*RI site in pVH51 (mini-ColE1[21]). A *Hin*dIII site is located in the initial portion of *E. coli trp*(*G*)*D*[22]. The exact location of this restriction site, 275 base pairs from the *trpE–trp*(*G*)*D* punctuation region, has been determined by DNA sequence analysis (C. Yanofsky *et al.*, in preparation). The molecular weight of 23,000 for the amino terminal proteolytic fragment of *E. coli* AS component II[6] is very similar to that of component II from *S. marcescens* (21,000). By analogy to the *S. marcescens trp* operon we would therefore expect the *trpG-D* junction region in *E. coli* to be located approximately 300 base pairs downstream from the *Hin*dIII site. A restriction map of the corresponding DNA segment is shown in Fig. 1*b*. DNA sequence analysis was performed with DNA fragments labelled at either the *Hpa*II site at position 225 or the *Pvu*II site at position 380 as indicated in Fig. 1*b*. The nucleotide sequence of this DNA segment is presented in Fig. 2 along with the sequence of the analogous region from *S. marcescens*.

The *S. marcescens* nucleotide sequence contains a potential ATG translation initiation codon at positions 88–90 (Fig. 2). The predicted amino acid sequence from this site is identical to the previously determined amino acid sequence at the N-terminal end of *S. marcescens* PRT[23]. This confirms our assumption that *trpD* follows *trpG* in the *S. marcescens trp* operon. The amino acid sequence at the C-terminal end of *S. marcescens* AS component II was determined by carboxypeptidase digestion of purified component II. The results [(Trp, Ala)-Leu-Ala-Lys-COOH] indicate that the TAA at positions 70–72 is used as the translation stop codon. In *S. marcescens trpG* and *trpD* are thus separated by an untranslated region 16 base pairs in length. Six contiguous mucleotides within this region (TAAGGA) are homologous to the 3′ terminus of *E. coli* 16S rRNA[24], a feature common to many prokaryotic ribosome binding sites[25].

The nucleotide and amino acid sequences preceding and following the segment corresponding to the *trpG–trpD* intercistronic region have been extensively conserved between *S. marcescens* and *E. coli*. Twenty of 23 amino acids in the *trpG* portion and 16 of 22 amino acids in the *trpD* portion are identical (Fig. 2). Several changes at the nucleotide level (eight in *trpG*; seven in *trpD*) are not reflected at the amino acid level. In contrast to this, sequence conservation is not immediately apparent in the *trpG–trpD* intercistronic region (Fig. 2). In *E. coli* 15 nucleotides are present in this DNA segment compared with 16 in *S. marcescens*; as a consequence the coding sequences of the *E. coli trpG* and *trpD* segments are in phase. This, together with the absence of translational stop and start signals (also there is no obvious sequence homology to the 3′ end of 16S ribosomal RNA), accounts for the production of a 'fused' *trp*(*G*)*D* polypeptide in *E. coli*.

The marked similarity between the two sequences suggests a common origin. This implies that at least once during the evolution of the *trp* genes in the Enterobacteriaceae a gene fusion or gene separation event occurred in the *trpG–trpD* region. Bonner *et al.*[26] have suggested that evolutionary development progresses from independent proteins through multimeric enzymes to fused gene products. Alternatively, as pointed out by Largen *et al.*[27], a fusion event involving ancestral *trpG* and *trpD* genes may have occurred before the appearance of the Entrobacteriaceae, in which case the separate *trpG* and *trpD* genes of *S. marcescens* would represent the result of a subsequent separation of the fused genes. Although the first possibility seems more plausible, the available evidence does not allow us to rule out the alternative route.

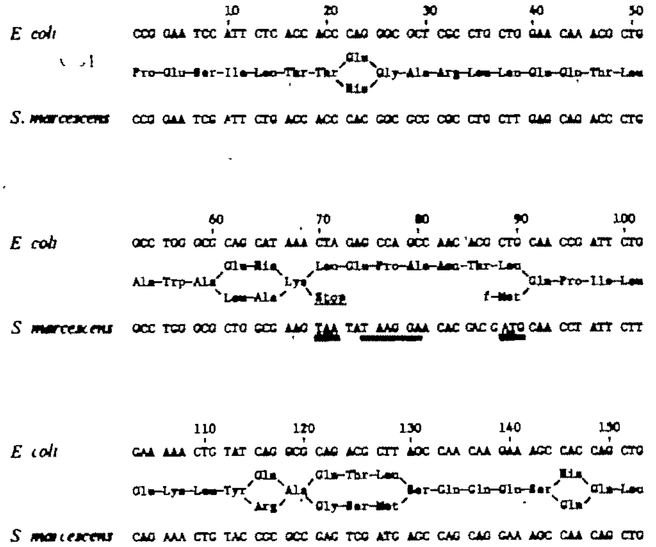
10
20
30
40
50
E coli
CCG GAA TCC ATT CTC ACC ACC CAG GGC GCT CGC CTG CTG GAA CAA ACG CTG
Pro-Glu-Ser-Ile-Leu-Thr-Thr-Gln/His-Gly-Ala-Arg-Leu-Leu-Gln-Gln-Thr-Leu
S. marcescens
CCG GAA TCG ATT CTG ACC ACC CAC GGC GCC CGC CTG CTT GAG CAG ACC CTG
60
70
80
90
100
E coli
GCC TGG GCG CAG CAT AAA CTA GAG CCA GCC AAC ACG CTG CAA CCG ATT CTG
Ala-Trp-Ala-Gln-His/Leu-Ala-Lys-Leu-Gln-Pro-Ala-Asn-Thr-Leu/Stop ... f-Met-Gln-Pro-Ile-Leu
S marcescens
GCC TGG GCG CTG GCG AAG TAA TAT AAG GAA CAC GAC G ATG CAA CCT ATT CTT
110
120
130
140
150
E coli
GAA AAA CTG TAT CAG GCG CAG ACG CTT AGC CAA CAA GAA AGC CAC CAG CTG
Glu-Lys-Leu-Tyr-Gln/Arg-Ala-Gln-Thr-Leu/Gly-Ser-Met-Ser-Gln-Gln-Glu-Ser-His/Gln-Gln-Leu
S marcescens
CAG AAA CTG TAC CGC GCC CAC TCG ATG AGC CAG CAG GAA AGC CAA CAG CTG

Fig. 2 Nucleotide sequence of the DNA region surrounding the *trpG–trpD* interconnecting/intercistronic region in *E. coli* and *S. marcescens*. The two sequences have been lined up in the *trpG* and *trpD* portion to show homologies. The predicted amino acid sequence is indicated between the DNA sequences. A split amino acid sequence indicates that the nucleotide sequence predicts a different amino acid for the two organisms at that position. The predicted amino acid sequence following the ATG codon at position 88–90 of the *S. marcescens* nucleotide sequence corresponds exactly to the previously determined amino acid sequence at the N-terminal end of *S. marcescens* PRT[23]. The C-terminal residues of *S. marcescens* AS component II were determined by carboxypeptidase A and B digestions of the purified protein[8,33]. The sequential appearance of Lys, Ala, Leu, and Trp served to identify the TAA at position 70–72 as the translation stop codon. The message punctuation signals in the *S. marcescens trpG–D* intercistronic region (translation stop and start codons, homologies to the 3′ end of *E. coli* 16S rRNA) are underlined. DNA sequence analysis was performed by the method of Maxam and Gilbert[36]. Details of this and related procedures such as isolation of plasmid DNA and DNA restriction fragments, restriction site mapping, phosphatase treatment, polynucleotide kinase reactions and agarose and polyacrylamide gel electrophoresis are given in refs 38, 39.

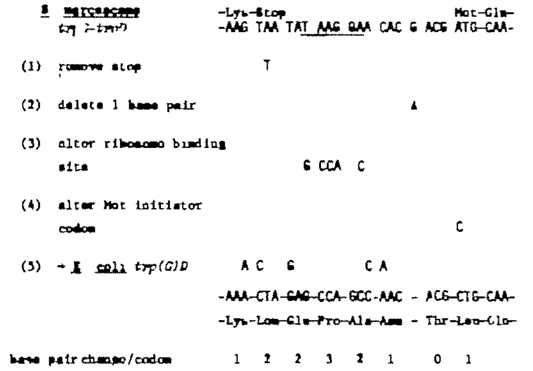
S marcescens
trpG-trpD
-Lys-Stop
Met-Gln-
-AAG TAA TAT AAG GAA CAC G ACG ATG-CAA-
(1) remove stop
T
(2) delete 1 base pair
A
(3) alter ribosome binding site
G CCA C
(4) alter Met initiator codon
C
(5) → E coli trp(G)D
A C G C A
-AAA-CTA-GAG-CCA-GCC-AAC - ACG-CTG-CAA-
-Lys-Leu-Glu-Pro-Ala-Asn - Thr-Leu-Gln-
base pair change/codon
1 2 2 3 2 1 0 1

Fig. 3 Hypothetical sequence of events involved in the conversion of the separate *trpG* and *trpD* genes of *S. marcescens* into the single fused *trp*(*G*)*D* gene of *E. coli*. In reality the postulated fusion event would have taken place in a common ancestor of both organisms which may have been similar to *S. marcescens* in its nucleotide sequence.

If the fused *trp*(*G*)*D* arrangement of *E. coli* were ancestral to the *S. marcescens* gene order, the conversion of the first Lys codon of Fig. 3 to a stop codon would probably suffice to give separate, active, *trpG* and *trpD* polypeptides. The present day *trpG–trpD* interconnecting nucleotide sequence of *E. coli* may in fact have weak ribosome binding and translation initiation activity. Mutational studies suggest that there is a translation reinitiation site within *E. coli trp*(*G*)*D* which can produce a polypeptide with PRT activity[10,12]. In addition, as mentioned earlier, the polypeptide segment specified by the *trpG* portion of *trp*(*G*)*D* of *E. coli* has GAT activity. Thus separation could occur without loss of activity. Subsequent mutational changes could improve the efficiency of ribosome binding to the initiation region of *trpD* and consequently increase the yield of PRT.

One of several hypothetical sequences of events that would result in the formation of the fused *E. coli trp*(*G*)*D* gene from the separate *trpG* and *trpD* genes of *S. marcescens* is illustrated in Fig. 3. In a first step a single mutational event removes the *trpG* translational stop signal, allowing out-of-phase translation into the adjacent *trpD*. Both *trpG* and *trpD* are still expressed but out-of-phase translation probably would reduce production of the *trpD* polypeptide. Deletion of a single base pair in the immediately adjacent region (step 2) results in the formation of a fused polypeptide whose two functional domains would be held together by an 'immature' connector region. However, the fused gene retains the efficient parental *trpD* ribosome binding site and thus might produce both a fused *trp*(*G*)*D* polypeptide and a *trpD* polypeptide. Subsequent mutations in the intercistronic region could inactivate the ribosome binding site and/or introduce functionally acceptable amino acids (step 3) and alter the ATG start sequence (step 4). Three synonymous codon changes and two substitutions resulting in amino acid replacements (step 5) would then give the *E. coli* sequence. The *E. coli* connector region presumably does not interfere with the correct folding of the two functional domains of the fused polypeptide. As can be seen from the last line of Fig. 3, the overall sequence of events would not involve extensive base changes in the intercistronic region. This sequence of events neglects the fact that the fusion event would have taken place in an unknown *Serratia*-like ancestor which differed from *S. marcescens* in its nucleotide sequence. It is obvious that a single deletion event could more simply fuse the adjacent *G* and *D* genes. Perhaps the connector region of *E. coli*, in mRNA or protein, has some important unsuspected function.

Gene fusion and separation may have occurred several times in the evolution of the tryptophan genes. In yeast the third and fourth steps of the biosynthetic pathway are catalysed by separate proteins coded for by unlinked genes[28], while in *E. coli* and many other prokaryotes both reactions are catalysed by a single bifunctional polypeptide encoded in a single gene, *trpC*[1,28,29,30]. The converse is found for tryptophan synthetase, where the fungal enzyme consists of a fusion of α and β chain elements[31,32] which are separate polypeptides in *E. coli* and other prokaryotes[33]. The *trpG–trpD* case may be unique in that both the fused and separate states of the genes are found within closely related organisms, where evolutionary divergence apparently has not been significant enough to totally obscure the original fusion event.

If the fusion of genes involved in the same pathway confers a selective advantage, one wonders why in *S. marcescens* the separate *trpG* and *trpD* genes have not been replaced by a fused genetic element, particularly since fusion need involve so few mutational events. It is conceivable that in order for the fusion to become fixed, compensatory mutations must occur elsewhere within *trpG* or *trpD*. In such circumstances, the probability of a successful fusion event would be substantially reduced. Alternatively, *S. marcescens* AS component II may serve as a GAT subunit in *p*-aminobenzoate formation as does AS component II of *B. subtilis*[16,17] and *Acinetobacter calcoaceticus*[34]. Such a dual function of the *trpG* gene product would obviously place severe restrictions on the likelihood that *trpG* would undergo gene fusion.

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Specific binding of Mu repressor to DNA

PHAGE MU is a temperate virus of *Escherichia coli* (for reviews see refs 1, 2). The immunity system of Mu has been shown by genetic and physical mapping to lie in the furthest left, 1,000-base-pair restriction fragment of the phage DNA[3-6]. This end of the phage genome is associated with a short (100-base pair) segment of bacterial DNA[4]. The *Hin*dIII-c restriction fragment of Mu (the furthest left 1,000 base pairs[4]) has been cloned into the amplifiable plasmid pMB9 (ref. 7). These recombinant plasmids confer high levels of Mu immunity on their hosts and, when recloned into minicell-producing strains, were shown to produce an additional protein of 25,000 subunit molecular weight in purified minicells[6,7]. This protein is assumed to be the Mu repressor because its production is correlated with immunity to Mu. We report here experiments which show that this protein binds specifically and with high affinity to Mu DNA, as would be expected of the Mu repressor. Furthermore, we demonstrate that the DNA sequence (operator) to which the Mu repressor binds is located on the same *Hin*dIII-c restriction fragment of Mu DNA as the repressor gene.

In these studies DNA binding was assayed using the unpurified crude extract of labelled minicells as a source of repressor. These extracts contain so few other labelled proteins that the only purification required is the removal of the plasmid DNA. Extracts of minicells derived from strains transformed

Table 1 Specific binding of the temperature-sensitive Mu repressor to Mu DNA

Crude lysate of repressor	DNA-bound repressor (fractions 5/6 (6/7*))	Unaggregated repressor (fraction 9/10)
−DNA	<1%	31%
+Mu DNA	13%	26%
+D108 DNA	<1%	31%
+λ DNA	<1%	31%
+25 DNA	14%*	23%
+p5D7 DNA	<1%	7%

The crude lysate of minicells was prepared as described in the legend to Fig. 1, and the top fraction of the high salt gradient used as the source of repressor. Aliquots were mixed with buffer IV or buffer IV plus 20 μg Mu, λ or D108 DNA, or 2.0 μg linearised plasmid 25 DNA or plasmid p5D7 DNA. The DNA concentrations were such that approximately equal numbers of DNA molecules were added to each binding mix. The binding mixture was incubated and run on gels as described in Fig. 1 legend. The fractions collected were assayed for proteins and DNA as before. Autoradiographs of the SDS-acrylamide gels run on gradients with repressor alone, repressor + Mu, repressor + D108 and repressor + λ DNA are shown in Fig. 1. The autoradiographs were scanned and the peaks of repressor weighed and normalised as described in Fig. 2 legend. The per cent of total repressor recovered from fractions 5+6, or 6+7 (DNA-bound repressor), and from fractions 9+10 (unaggregated repressor) is shown. Due to the noise in the system, repressor in amounts <1% of the total repressor in the gradient would not be detectable by scanning; Mu, D108, λ and plasmid p5D7 DNA sediment to fractions 5 and 6 in the gradients, whereas plasmid 25 DNA linearised by digestion with *Hin*dIII sediments to fractions 6 and 7 in these conditions.

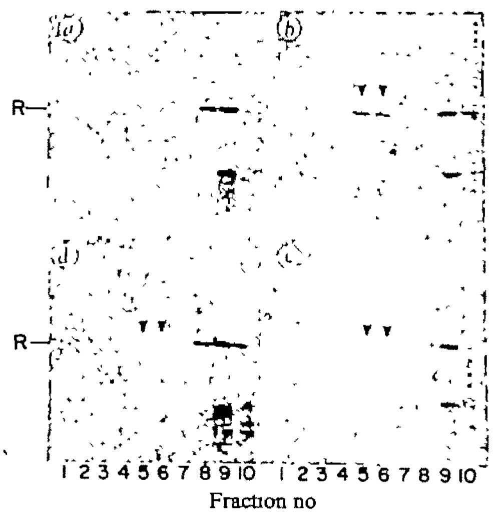

Fig. 1 Comparison of repressor binding to Mu, D108 and λ DNA ^{35}S-Methionine-labelled minicells purified (according to the procedure described by Zipser *et al*[6]) from χ1274/pMB9-mu *cI*ts62 *Hin*dIII-c 25 (frozen in 10 mM Tris, *p*H 8, 5 mM EDTA, 10% sucrose, 0.05 M NaCl) were lysed in the following manner The minicells were thawed slowly on ice, and NaCl and lysozyme were added to final concentrations of 0.3 M NaCl and 150 μg ml^{-1} lysozyme. After 1 h on ice, the minicells were frozen in a dry ice–ethanol bath and thawed on ice, five times. Triton X-100 was added to a final concentration of 0.33% (v/v), and the minicells were incubated for 20 min at 0 °C. The crude lysate (150 μl total volume) was centrifuged at 20,000 r.p.m. in a Beckman SW 60 rotor to remove cell debris and insoluble proteins. The supernatant was adjusted to 0.6 M NaCl and layered onto a 5 ml 5–20% sucrose in buffer IV (10 mM Tris, *p*H 8, 1 mM EDTA, 0.1 mM dithiothreitol, 50 μg ml^{-1} bovine serum albumin) gradient containing 0.6 M NaCl. The gradient was then centrifuged for 2 h at 45,000 r.p.m. (4 °C) in the Beckman SW 50.1 rotor, and 10 0.5-ml fractions were collected from the bottom of the gradient. Sedimentation through high salt was done to eliminate variability in the binding assay due to repressor binding to endogenous DNA In these conditions, the repressor and other labelled proteins remain at the top of the gradient, whereas endogeneous DNA moves to the middle of the gradient. The top fraction was used as the source of repressor in the binding assay. Samples (0.1 ml) of the top fraction were mixed with buffer IV or buffer IV plus 20 μg DNA (Mu, D108 or λ), and adjusted to 0.2 M NaCl. The binding mixtures were incubated for 30 min at 0 °C and then layered onto 5 ml 5–20% sucrose in buffer IV gradients containing 0.05 M NaCl. The gradients were centrifuged for 2 h at 45,000 r.p.m. (4 °C) in a Beckman SW 50.1 rotor, and 10 0 5-ml fractions were collected from the bottom of each gradient. Samples from each fraction were subjected to electrophoresis on 17.5% SDS–polyacrylamide gels. The gels were autoradiographed to assay for labelled proteins. Samples from the gradient fractions were also subjected to electrophoresis on 1% agarose gels. These gels were stained with ethidium bromide and photographed under UV light to assay for DNA Autoradiographs of the SDS-polyacrylamide gels run on gradients with *a*, repressor alone; *b*, repressor plus Mu DNA; *c*, repressor plus D108 DNA; and *d*, repressor plus λ DNA are shown above. The arrow labelled R indicates the position of the repressor band on the gels The vertical arrows indicate the position of the DNA in the gradients. Mu and λ DNA were prepared from lysogens carrying Mu *cI*ts62 and λ *cI*857 S7, respectively, by the procedure of Kahmann[12] D108 was prepared in a similar manner, except that purification of phage was done after infection of strain 783 (Thi$^-$, Trp$^-$, su$^+$) with wild-type D108.

with plasmids carrying the Mu repressor gene were mixed with unlabelled DNA and then sedimented through sucrose gradients[8,9]. To assay repressor, samples from each fraction of the gradients were subjected to electrophoresis on SDS-polyacrylamide gels, and the amount of repressor determined from autoradiographs of these gels. The position of the DNA in the gradient is determined from ethidium bromide-stained agarose gels run on each gradient fraction.

The specificity of Mu repressor is demonstrated in Fig. 1 and Table 1. When a repressor extract without DNA is sedimented through a gradient, the repressor, as well as other labelled proteins, remain near the top of the gradient. When the same extract is used with Mu DNA, a significant fraction of the repressor sediments with the DNA, but the position of other labelled proteins remains unchanged. If equal amounts of DNA from D108, a phage heteroimmune to Mu, or from phage λ are used, only trace amounts of Mu repressor are bound.

When the binding was carried out at 0.1 M rather than 0.2 M NaCl, some repressor was associated with D108, but less than the amount associated with Mu DNA. More repressor was found in the fractions between the DNA peak and the top of the gradient with D108 DNA than was found with Mu DNA, suggesting that the repressor was dissociating from D108 DNA as the DNA sedimented through the gradient. This indicates that although repressor binds tightly to Mu DNA, it has some affinity for other DNAs at lower salt concentrations.

Increasing the salt concentration in the binding reaction from 0.1 to 0.3 M NaCl had little effect on the amount of repressor bound to Mu DNA, but increasing it to 0.6 M NaCl eliminated repressor binding to Mu DNA. Binding was also not detected when both the binding mixture and the gradient contained 0.2 M NaCl.

Phage D108 is closely related, but heteroimmune, to phage Mu[10]. Heteroduplex analysis of D108 and Mu shows a total nonhomology of 6% between the two phages, the largest region of nonhomology (3%) being in the left end of the phages[10], where the repressor maps. As D108 does not bind Mu repressor, the operator must be nonhomologous and could be in the left end of the phage genome also. This would be similar to the situation found with bacteriophage λ, where the operators to which the λ repressor binds are located close to the repressor gene[8,11]. This might mean that the Mu operator is also on the cloned *Hin*dIII-c fragment that carries the repressor gene.

To test this possibility DNA of plasmid 25 (the same plasmid used to synthesise repressor) was used in the binding assay. As shown in Fig. 2, in the same conditions which gave specific binding to Mu DNA, the repressor binds to linearised plasmid 25, but not to linearised pMB9 DNA. Furthermore, the repressor does not bind to DNA of plasmid p5D7 (a recombinant plasmid constructed by the terminal transferase tailing method in a fashion analogous to that of plasmid 25 but inserting yeast genes instead of Mu DNA). This argues against the possibility that the repressor binds to the AT-rich regions created during cloning (see Table 1). Three other plasmids (36, 42 and 54 (ref. 7)) which were constructed in the same manner as plasmid 25 and which carry the same *Hin*dIII fragment of Mu *cI*ts62, also show specific binding of the Mu repressor. Thus, an operator to which the Mu repressor binds, is located in the furthest left 1,000 base pairs of the Mu genome.

The crude repressor preparations used for these studies contain varying amounts of inactive repressor (repressor which cannot bind to operator-containing DNA). The amount of

Table 2 Binding of the temperature-sensitive Mu repressor to varying amounts of DNA

Crude lysate of repressor	DNA-bound repressor (fractions 6/7)	Unaggregated repressor (fractions 9/10)
−DNA	<1%	48%
+0.1 μg 25 DNA	28%	44%
+0.5 μg 25 DNA	55%	47%
+2.5 μg 25 DNA	81%	38%
+12.5 μg 25 DNA	62%	35%
+0.5 μg pMB9 DNA	9%	49%

A crude lysate of minicells was prepared as described in Fig. 1 legend. Aliquots of the top fraction of the high salt gradient were mixed with buffer IV, or buffer IV plus varying amounts of linearised plasmid 25 DNA or 0.5 μg linearised plasmid pMB9 DNA, and adjusted to 0.2 M NaCl. Binding mixtures were incubated and run on gradients as before. The fractions collected were assayed for proteins and DNA as described in Fig. 1 legend. Autoradiographs of the acrylamide gels were scanned for the repressor band and the peaks weighed and normalised as described in Fig. 2 legend. The per cent of total repressor recovered in fractions 6+7 (DNA-bound repressor) and in fractions 9+10 (unaggregated repressor) is shown above. Due to the noise in the system, repressor in amounts <1% of the total would not be detectable by scanning. The DNA sediments to fractions 6 and 7 in these conditions.

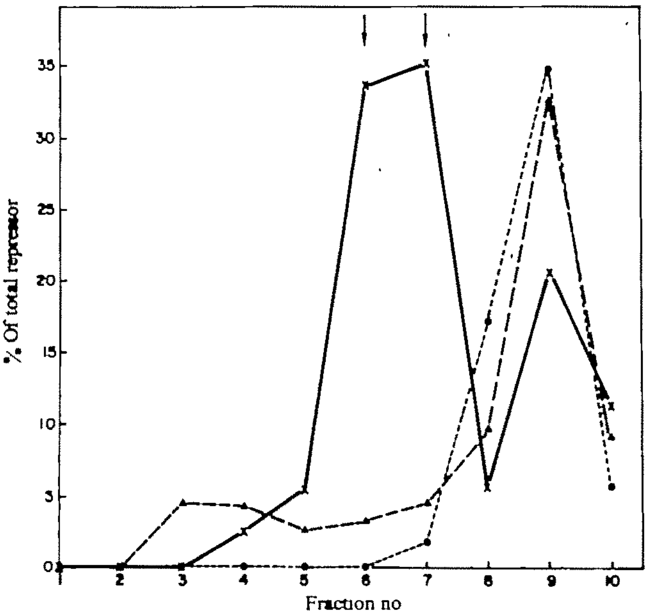

Fig. 2 Comparison of repressor binding to plasmid 25 and pMB9 DNA. A crude lysate of minicells was prepared as described in the legend to Fig. 1. As before, the top fraction of the high salt gradient was used as the source of repressor. Aliquots were mixed with buffer IV or buffer IV plus 2.5μg of linearised plasmid 25 or pMB9 DNA, and adjusted to 0.2 M NaCl. The binding mixture was incubated and run on gradients as described in Fig. 1 legend. The fractions collected were assayed for proteins and DNA as before. The repressor band in autoradiographs of the acrylamide gels were scanned using a Joyce, Loebl densitometer (Chromoscan Mk. II.). The peaks were cut out and weighed to determine the amount of repressor in each fraction. To normalise the amount of repressor in each fraction, samples of the binding mixtures before sedimentation were subjected to electrophoresis on 17.5% SDS–polyacrylamide gels. Autoradiographs of these gels were scanned for the repressor band and the peaks cut out and weighed. The amount of repressor in the control was multiplied by dilution factors to determine the amount of repressor in each binding mix. As an equal amount of the repressor preparation was used for each binding mixture, an average of the amount of repressor in the controls was used to normalise the amount of repressor in fractions of the gradients. The per cent of the total repressor recovered in fractions of gradients with *a*, repressor alone (●), *b*, repressor plus plasmid 25 DNA (×), and *c*, repressor plus pMB9 DNA (△) is shown. The vertical arrows indicate the position of DNA in the gradients. DNA of both plasmid 25 and pMB9 was digested with the restriction enzyme *Hin*dIII (Bethesda Research Labs) which cleaves both DNAs once, making linear molecules. Uncut plasmid 25 also shows binding of the repressor, however, two peaks of bound repressor are detected, corresponding to DNA in the monomer and dimer covalently closed circular forms. In these conditions the dimer DNA sediments further down the gradient than the monomer form. Plasmid 25 and pMB9 were purified from strain χ1274 (*E. coli* K12, Thi^-, Thr^-, Leu^-, $LacY^-$, $MalA^-$, MtL^-, Xyl^-, Ara^-, Gal^-, Str A, ton A, λ^R, azi, Min^-, su^+) according to the procedure of Clewell[13].

repressor found in the top fraction of the gradients did not decrease significantly with increasing amounts of DNA (Table 2). The amount of inactive repressor in any one preparation increased with the storage time of minicell preparations before use.

As shown in Table 2, both the amount of DNA-bound repressor and the overall amount of repressor recovered increase with increasing concentrations of plasmid 25 DNA, except at very high concentrations of DNA. Similarly, more repressor was recovered when pMB9 DNA was added to the same preparation, than was recovered after sedimenting the preparation alone. In this case, however, the repressor was not associated with pMB9 DNA, but was spread throughout the gradient (Fig. 2). This suggests that the active repressor proteins will bind to each other, if not bound to DNA, forming aggregates which sediment rapidly in the gradient. Thus, if the repressor extract is sedimented without added DNA, much of the active repressor goes to the bottom of the gradient.

This repressor–repressor interaction must be weaker than repressor–DNA binding, as any DNA added to the repressor seems to inhibit aggregate formation. As the repressor dissociates from non-operator-containing DNA (that is, pMB9) during sedimentation, however, it will begin to form repressor–repressor aggregates, and this repressor is detected throughout the gradient. Repressor binding to operator-containing DNA (plasmid 25 DNA) is tight, so that very little repressor dissociates from the DNA during sedimentation and no aggregation of the repressor occurs. This finding is consistent with the observation that there is a decrease in the amount of total repressor recovered in binding assays carried out at 0.3 M rather than 0.1 M NaCl. The decrease in recovery is more dramatic with D108 or pMB9 DNA than with Mu or plasmid 25 DNA.

Further characterisation of the nature of the binding of Mu repressor to Mu DNA (and repressor–repressor interaction) requires purification of significant amounts of repressor protein. This is feasible because large quantities of repressor can be made in strains carrying plasmid 25 (or similar recombinant plasmids), and crude DNA binding assays (like that described above) allow easy detection of active repressor during purification.

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Surface and inside volumes in globular proteins

MEASUREMENTS of the surface area accessible to solvent provide a convenient definition of the surface and the inside volumes in proteins of known X-ray structure. The study of the accessibility to solvent of amino acid residues in several proteins[1–3] has confirmed the early observation that polar residues are found mostly on the surface and non-polar residues mostly inside globular protein structures. But the accessibility shows systematic variations with the molecular weight, because of the change in surface to volume ratio. Experimental data[4] indicate that the accessible surface area A (in Å^2) of monomeric globular proteins follows the law[5,6]:

$$A = 11.1\, M^{2/3} \qquad (1)$$

which implies that the mean accessible surface area per residues decreases like $M^{-1/3}$ (where M is molecular weight) with increasing M, from about 68 Å^2 for proteins of 6,000 molecular weight to 38 Å^2 for proteins of 35,000 molecular weight. Thus, the accessibility to solvent is not a characteristic of the amino acids.

We made histograms of the accessible surface areas of residues in a sample of 22 proteins, and found that the number n_B of buried residues, defined as having less than a given accessible surface area A_m, varies with the total number n of residues in the

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polypeptide chain according to:

$$n^{1/3} - n_B^{1/3} = k \tag{2}$$

where k depends only on A_m. Completely buried residues (zero accessible surface area) are few: 4 in pancreatic trypsin inhibitor (58 residues), 40 in carboxypeptidase A (307 residues). Better statistics are obtained by choosing $A_m = 10$ to 20 Å^2, which amounts to consider as buried residues which have one or two atoms in contact with the solvent. With $A_m = 20$ Å^2, the average value and standard deviation of k are 1.62 ± 0.13. The geometrical significance of equation (2) is apparent in Fig. 1: k represents the thickness of the surface layer made of residues in contact with the solvent. While the hard sphere model of globular proteins, first presented by Fisher[7], is in formal agreement with equations (1) and (2), the fit only implies that the gross geometrical properties of globular proteins (surface and volume) are those of a set of solid bodies which deviate in similar ways from a spherical shape. This is not true of subunits in oligomeric proteins: the subunits of tetrameric lactate dehydrogenase[4] and glyceraldehyde-3-phosphate dehydrogenase have much larger accessible surface areas and fewer buried residues than expected from equations (1) and (2).

Table 1 gives the amino acid composition of our sample of buried and accessible residues. The buried residues make up the protein inside; 56% are Leu, Ile, Val, Ala, or Gly, another 22% are Phe, Ser, Thr or Cys, and 22% are of the 11 other types. The protein surface has a much more regular amino acid composition than the inside: non-polar residues are by no means absent, even though Asn, Asp, Glu, Gln, Lys and Arg constitute 40% of the accessible residues. The ratio f of the molar fractions of each amino acid in the two samples is of particular interest. It represents the partition coefficient of this amino acid between the surface and inside volumes. Consequently, $\Delta G_t = RT \ln f$ may be taken as the free energy of transfer from the inside to the surface. Though the exact value of f depends on the choice of A_m made in defining buried residues, the effect is small: reducing A_m from 20 Å^2 to 10 Å^2 changes the free energies by no more than their statistical standard deviation of 0.1–0.2 kcal mol^{-1}. Thus f and ΔG_t are characteristic of the amino acids in globular proteins.

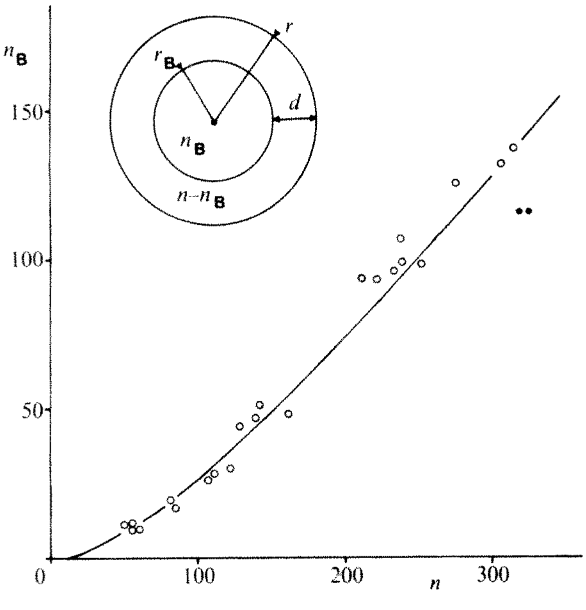

Fig. 1 Buried residues in globular proteins. The accessible surface areas of individual residues are calculated according to Lee and Richards[1] using a computer program of M. Levitt. The atomic coordinates are obtained from the Protein Data Bank, Cambridge, UK. The following 22 protein structures are used here and in Table 1: insulin, ferredoxin, rubredoxin, pancreatic trypsin inhibitor, high potential iron protein, cytochrome b_5, parvalbumin, cytochrome c_2, ribonuclease S, hen lysozyme, staphylococcus nuclease, flavodoxin, phage T4 lysozyme, papain, trypsin, concanavalin A, elastase, α-chymotrypsin, carbonic anhydrase B, substilisin, carboxypeptidase A, thermolysin. The number n_B of residues with less than $A_m = 20$ Å^2 of accessible surface area is plotted against the total number n of residues in the polypeptide chain (open circles). Filled circles represent the subunits of lactate dehydrogenase and glyceraldehyde -3-phosphate dehydrogenase. The insert represents the protein as made of two concentric spheres, the volumes of which are proportional to n and n_B; the radii are proportional to $n^{1/3}$ and $n_B^{1/3}$, and therefore k is proportional to the thickness d of the surface layer, made of $n - n_B$ accessible residues. For the 22 proteins: $A_m = 0$ Å^2, $k = 3.0 \pm 0.4$ (s.d.), $d = 9.6$ Å; $A_m = 10$ Å^2, $k = 2.0 \pm 0.2$, $d = 6.4$ Å, $A_m = 20$ Å^2, $k = 1.62 \pm 0.13$, $d = 5.2$ Å. The solid line is calculated from equation (2) with $k = 1.62$.

Table 1 Amino acid composition of the inside and surface

Residue	Molar fraction Buried	Accessible	Free energy (kcal mol^{-1}) f	ΔG_t	ΔG_h
Leu	11.7	4.8	2.4	0.5	1.8
Val	12.9	4.5	2.9	0.6	1.5
Ile	8.6	2.8	3.1	0.7	
Phe	5.1	2.4	2.2	0.5	2.5
Cys	4.1	0.9	4.6	0.9	
Met	1.9	1.0	1.9	0.4	1.3
Ala	11.2	6.6	1.7	0.3	0.5
Gly	11.8	6.7	1.8	0.3	0.0
Trp	2.2	1.4	1.6	0.3	3.4
Ser	8.0	9.4	0.8	−0.1	−0.3
Thr	4.9	7.0	0.7	−0.2	0.4
His	2.0	2.5	0.8	−0.1	0.5
Tyr	2.6	5.1	0.5	−0.4	2.3
Pro	2.7	4.8	0.6	−0.3	
Asn	2.9	6.7	0.4	−0.5	
Asp	2.9	7.7	0.4	−0.6	
Gln	1.6	5.2	0.3	−0.7	
Glu	1.8	5.7	0.3	−0.7	
Arg	0.5	4.5	0.1	−1.4	
Lys	0.5	10.3	0.05	−1.8	

Molar fractions (%) of the 20 amino acids in samples of 2,001 buried residues (less than 20 Å^2 accessible surface area) and 3,220 accessible residues (more than 20 Å^2); f is the ratio of the buried/accessible molar fractions; $\Delta G_t = RT \ln f$. ΔG_h is the free energy of transfer from an organic solvent to water[8]. Cys residues with a free −SH group are few in the sample; our data are more representative of substituted −SH groups (disulphides, metal adducts or thioethers).

In a protein of n residues, the most probable fraction g of buried residues of one type, say valine, is easily calculated from the data of Table 1:

$$g = \frac{f n_B}{n - n_B + f n_B} \tag{3}$$

where n_B is given by equation (2). Thus, the fraction of valines which are buried increases from 0.4 to 0.7 when the chain length increases from 60 to 300 residues, while it remains less than 0.1 for lysines.

Chothia[3] has noted that the average accessibility of residues in proteins is not correlated to the free energies ΔG_h of transfer from an organic solvent to water, measured by Nozaki and Tanford[8]. This is seen clearly in Table 1: ΔG_t is governed by the presence or absence of oxygen and nitrogen atoms in the side chain[1,3]. These polar atoms must find hydrogen bond partners when the residue is buried, a stringent requirement except for Ser and Thr residues, the hydroxyl group of which can easily form a hydrogen bond to adjacent main chain carboxyl groups[9]. Proline appearas as polar; its imino nitrogen cannot participate in normal main chain hydrogen bonds and behaves as a part of the side chain.

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Dimeric association of band 3 in the erythrocyte membrane demonstrated by protein diffusion measurements

BAND 3, a major constituent of the human erythrocyte membrane, comprises a class of membrane-spanning proteins which are implicated in anion and possibly other transport functions[1–4]. Various observations indicate that band 3 proteins may exist as dimers in the membrane[5–9]. The most direct indication has been obtained from chemical cross-linking experiments. The band 3 dimer is an early product of various cross-linking reactions[7], and the particular case of cross-linking by oxidative disulphide bridge formation results in almost quantitative dimerisation of band 3 (ref. 8). However, cross-linking experiments are in general inconclusive for they do not indicate whether a successful cross-linkage reflects a naturally occurring stable complex, or is the result of collisions between independent proteins which rapidly diffuse over small distances. In the recent elegant studies of Kiehm and Ji[9], rapid photochemical cross-linking was achieved by photolysing with a millisecond flash. Whilst these experiments undoubtedly provide the best evidence so far obtained for the existence of the band 3 dimer, they still leave some doubts concerning the possibility of collision-induced crosslinks. These can only be ruled out if the mean time between collisions is shown to be much longer than the lifetime of the flash-induced reactive species. The latter time was assumed to be short (of the order of 1 ms) on the basis of solution studies but was not measured in the experimental conditions. The collision frequency is a function of the local lateral diffusion coefficient. Band 3 diffusion over distances $>1\ \mu m$ seems to be slow in the erythrocyte membrane[10,11], but as discussed elsewhere[12], this may reflect restrictions to long range diffusion and does not rule out the possibility of more rapid local diffusion. If the local diffusion coefficient is in the range 10^{-9} to $10^{-10}\ cm^2\ s^{-1}$, the mean time between collisions is also of the order of 1 ms (ref. 9). A lateral diffusion coefficient in this range is consistent with the rotational diffusion of band 3 (ref. 12). Here we present a new physical approach to determining the self-association of band 3, based on the measurement of rotational diffusion. The rotational diffusion coefficient of an intrinsic membrane protein is strongly dependent on the diameter of its cross-section in the plane of the membrane. We show that cross-linking band 3 into dimers produces no observable decrease in the rotational diffusion coefficient of band 3 in the membrane. We therefore conclude that the dimer pre-exists in the membrane and is not a product of the cross-linking reaction following random collisions.

In the present experiments, the rotation of band 3 was measured by observing the flash-induced transient dichroism of a triplet probe, as previously described[12,13]. However, band 3 was labelled with eosin maleimide instead of the eosin isothiocyanate previously used; essentially similar results are obtained with either probe. Dimerisation of band 3 in the membrane was achieved by copper–phenanthroline-catalysed oxidation of cytoplasmic sulphhydryl residues to form disulphide bridges, as described by Steck[8].

Figure 1 shows the result of the cross-linking reaction as visualised by SDS-polyacrylamide gel electrophoresis. Band 3 has disappeared from its normal position in the gel, moving to a new band with a molecular weight of approximately 180,000, corresponding to the band 3 dimer. This result is identical to that reported by Steck[8].

The rotational mobility of band 3 in the cross-linked membranes was measured and compared with that of untreated controls. The transient dichroism measurements were analysed by calculating the absorption anisotropy $r(t)$ given by

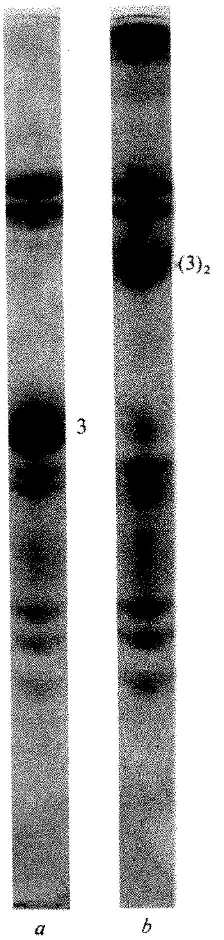

Fig. 1 Dimerisation of band 3 by oxidative disulphide bridge formation. Intact human erythrocytes were labelled with eosin-5-maleimide as described previously for other eosin derivatives[12,13,19], except that incubation was for 1 h at room temperature. These conditions yield approximately 1 molecule of bound eosin per band 3 monomer. Ghosts were prepared by hypotonic lysis[12] and given a final wash in 5 mM sodium phosphate, *p*H 8.0. They were allowed to react for 1 h at room temperature with one volume of *o*-phenanthroline (200 μM) and copper sulphate (40 μM) in the same buffer. The reaction was ended by the addition of 30 volumes 1 mM EDTA and 5 mM sodium phosphate, *p*H 8.0, and by a further wash in 5 mM sodium phosphate, *p*H 7.4. The control sample was subjected to the same procedures but without the addition of the copper–phenanthrolin reagent. Ghosts were then dissolved for electrophoresis and run on cylindrical 4% polyacrylamide gels in 0.2% SDS as described by Steck[8]. 3 and $(3)_2$ denote the positions of the band 3 monomer and dimer, respectively. Gel *a*, control; gel *b*, cross-linked ghosts.

$$r(t)=\frac{A_{\parallel}(t)-A_{\perp}(t)}{A_{\parallel}(t)+2A_{\perp}(t)} \qquad (1)$$

where $A_{\parallel}(t)$ and $A_{\perp}(t)$ are, respectively, the absorbance changes at time t after the flash for light polarised parallel and perpendicular with respect to the polarisation of the exciting flash. Figure 2 shows the time dependence of r for dimerised and untreated band 3. It is clear that the shapes of the two curves are virtually identical.

As discussed elsewhere[12,14], the predicted form of $r(t)$ for a protein which rotates only about the axis normal to the plane of the membrane is

$$r(t)=A_1\exp(-D_{\parallel}t)+A_2\exp(-4D_{\parallel}t)+A_3 \qquad (2)$$

where $D_{\parallel}$ is the rotational diffusion coefficient and A_1, A_2, A_3 are constants. To make a quantitative comparison of the cross-linked and control samples, we made a least-mean-square fit of

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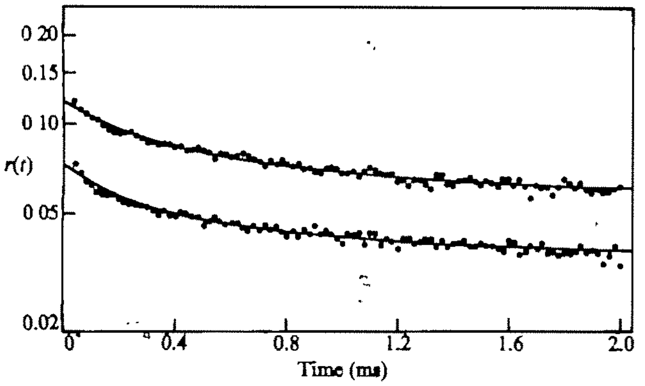

Fig. 2 Time dependence of the absorption anisotropy for untreated and cross-linked erythrocyte membranes. Flash-induced transient dichroism of the eosin probe was measured using a flash photolysis apparatus which is described in detail elsewhere[13]. The sample was excited at 540 nm by a linearly polarised flash of duration 1–2 µs. Absorbance changes arising from ground state depletion were measured at 520 nm. Upper curve, untreated control; lower curve, cross-linked as described in the legend to Fig. 1. Experimental points were obtained by averaging 32 signals. The solid lines were obtained by fitting the data to equation (2). For illustrative purposes the two curves have been artificially separated by a vertical displacement of the lower curve by 20%. Otherwise the data almost exactly superimpose. Measurements were made at 37 °C in 5 mM phosphate buffer, *p*H 7.4, under argon.

the data to equation (2). In this way we obtain $D_{\parallel} = 1{,}296 \pm 99\ \mathrm{s}^{-1}$ and $1{,}492 \pm 202\ \mathrm{s}^{-1}$ for the control and cross-linked membranes, respectively.

If we regard band 3 as a cylinder of radius a immersed to a depth h in the membrane, then the coefficient for rotational diffusion about the membrane normal is given by[15]

$$D_{\parallel} = (kT)/(4\pi a^2 h\eta) \qquad (3)$$

where η is the membrane viscosity. Thus, the rotational diffusion coefficient depends on the square of the radius of the rotating species. Assuming that dimerisation increases the radius of the particle by a factor of 1.5–2, we thus expect a two- to fourfold difference in $D_{\parallel}$ between the monomer and the dimer. As we fail to observe any decrease in $D_{\parallel}$ following cross-linking, we conclude that the band 3 dimer exists as a stable complex in the erythrocyte membrane independent of chemical cross-linking.

A possible objection to this conclusion is that the cytoplasmic portion of band 3 on which the reactive SH-group is located[16], is attached by a flexible linkage to the major part of the protein whose rotation we observe. Hence, cross-linking in this position might not change the rotation of band 3 monomers. However, Ross and McConnell have reported[17] that a spin label bound to the same SH-group is 'immobilised' (on the conventional electron spin resonance time scale). This strongly suggests that the cytoplasmic fragment is rigidly attached to the bulk of the protein and is not an independently rotating entity.

It should also be pointed out that cross-sectional shapes of band 3 could in principle be devised which would considerably reduce the expected change in $D_{\parallel}$ on association of monomers into dimers. Because we could detect a change in radius with cross-linking as small as 15%, we consider this a rather improbable explanation of our data. Taken together with the earlier chemical studies, there can be little doubt that band 3 does exist as a stable dimer within the human erythrocyte ghost membrane. However, further association of band 3 dimers among themselves or with other integral membrane proteins such as the major sialoglycoprotein are not ruled out.

The functional significance of the band 3 dimer is unclear. As the major part of band 3 is the anion transport system[1,2,18], it is tempting to speculate that the anion channel is formed at the interface between the two subunits of the dimer. However, inhibition studies with disulphonic acid stilbene inhibitors[18] and eosin maleimide[19] show that about one inhibitor bound per band 3 monomer is required for total inhibition of anion transport. This suggests that there are two anion binding sites per channel, if there is only one channel per band 3 dimer. Alternatively, one half of the inhibitor molecules could be bound to nonspecific sites on the protein.

Thus, the experiments reported here demonstrate how rotational diffusion measurements can be used to detect stable oligomeric complexes of proteins within biological membranes. We anticipate that the approach may be extended to other membrane proteins, for example, the (Mg^{2+}, Ca^{2+})ATPase of the sarcoplasmic reticulum, where the possibility of self-association is now under consideration[20,21].

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Errata

In the review article 'Multiple receptors for dopamine' by J. W. Kebabian and D. B. Calne, *Nature* **277**, 93–96, line 27 in the right-hand column of page 95 should read 'activity, have been used to . . .' and line 34 should read 'studies are only just beginning to be . . .'

In the letter 'MHC matching shows that at least two T-cell subsets determine resistance to HSV', by E. L. Howes *et al.*, *Nature* **277**, 67–68, in line 10 in paragraph 6, for 'in the thymus' read 'in the remnant thymus'.

In the letter 'Loss of immunoreactivity in long-term bone marrow cultures' by T. M. Dexter and E. Spooncer, *Nature* **275**, 135–136 (1978), on page 136, lines 5 and 8 for H-v-G read G-v-H.

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matters arising

Coesite and stishovite in the Vredefort, South Africa

RECENTLY I have been involved in tectonophysical research in the Witwatersrand collar sequence of the Vredefort dome, concentrating on microfabric studies in the collar quartzites with particular reference to the shock metamorphic microstructures found in some of these rocks. These shock microstructures include planar features, crystallographically controlled cleavage, crystallographically controlled faults and mosaic extinction. Other microstructures include extreme undulose extinction and deformation bands.

Planar features are the most widely accepted indicators of shock deformation and in the Vredefort collar quartzites they occur as decorated, planar discontinuities parallel to crystallographic planes in the quartz. The crystallographic orientation of planar features can be used to estimate the magnitude of shock pressures sustained by the rock sample[1-3], and has been used with considerable success at the Charlevoix and Slate Islands structures[2,3]. The technique involves measuring the orientation of planar features in 20 to 30 grains in a sample, classifying each grain on the basis of its planar feature content[1], and assigning to each a pressure value calculated from experimental data[2,3]. The shock pressure in the rock is then calculated as the mean of shock pressures in individual grains.

Samples from the Witwatersrand collar (Fig. 1) have been used in the type of analysis described above. Shock estimates range from 59 to 148 kbar. A diagram showing estimated shock pressure as a function of distance from the core–collar contact is shown in Fig. 2 in which the curve of best fit is nonlinear and given by

$$P_{\mathrm{sh}} = 145.47D^{-0.39}$$

where P_{sh} is the estimated shock pressure and D is the distance from the core-collar contact. There is a strongly evident decrease in shock pressure away from the contact.

Many of the shock microstructures in the Lower Witwatersrand quartzites, however, have been recovered and recrystallised. The amount of recrystallisation increases towards the core, from minor recrystallisation away from the core (in which minor amounts of new quartz occur along planar features and grain boundaries) through major recrystallisation (where a large percentage of the rock is recrystallised with only relict primary grains), so that the Hospital Hill quartzites are completely recrystallised. Minor recrystallisation extends to the outermost quartzites of the collar. Major recrystallisation, however, occurs in a zone[4] which has the same shape as, but is not as extensive as, the limit of contact metamorphism in the collar (which is shown in Fig. 1), so that it is probable that recrystallisation in the quartzites is a result of the thermal metamorphism which produced the contact metamorphic metapelites in the collar.

Stöffler[5] has demonstrated that after their formation, coesite and stishovite are metastable and susceptible to reversion during thermal metamorphism. Certainly one would expect that large amounts of high pressure quartz polymorphs would be found in the lowermost Witwatersrand rocks (those closest to the core) since this is where the shock pressures are greatest (Fig. 2). However, as Martini has shown, none are found; samples from the Hospital Hill quartzite examined by Martini (those from Marra 395, Fig. 1 and Vlakspruit 1180, which is off Fig. 1 to the south-west) are barren. However, Martini did find coesite and stishovite at Weltevrede 167 and coesite at Ospan 238 which are high up in the Witwatersrand sequence (Fig. 1).

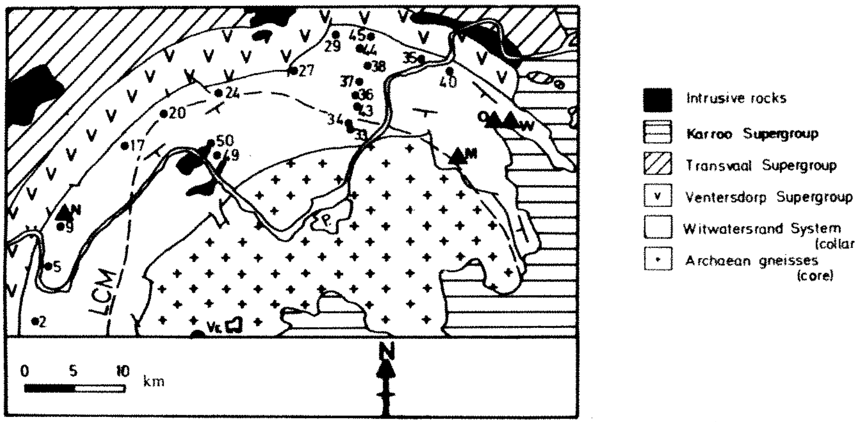

Fig. 1 A simplified geological map of the Vredefort dome (modified after Bisschoff[6]) showing the limit of metamorphism in the collar (LCM)[6] and the localities of samples used in the shock pressure analysis (dots). The approximate localities of farms on which Martini[7] took samples are shown as triangles where W is Weltevrede 167, O is Ospan 238, M is Marra 395 and N is Nooitgedacht 508. The towns of Parys (P) and Vredefort (Vr) are also shown.

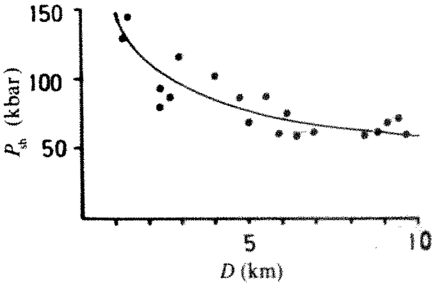

Fig. 2 A plot of estimated shock pressure (P_{sh}) against distance from the core–collar contact (D) for samples from the Vredefort collar sequence.

I suggest, therefore, that no polymorphs are found in the lowermost collar rocks because they were affected by the post-shock, metamorphic overprint. Only those rocks not strongly affected by metamorphism and/or recrystallisation (that is, the rocks further from the core) would retain their high pressure polymorphs of quartz.

The sample which Martini took on Nooitgedacht 508 (Fig. 1) is, however, barren. This is well outside the thermal aureole and does not fit comfortably into the model outlined above. My sample from Nooitgedacht 508 (sample 9, Fig. 1) shows evidence of minor amounts of recrystallisation along planar features; this indicates that these rocks were very slightly affected by the metamorphism. This may account for the lack of high pressure minerals in these rocks.

This work was supported by the CSIR and the South African National Geodynamics Project.

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MARTINI REPLIES—The microfabric investigations conducted by Lilly are of great interest as they not only confirm the very high pressure conditions having characterised the Vredefort Dome event, but also give a geographical distribution of these conditions. However, the number of samples analysed for coesite and stishovite is statistically not representative and at this stage it is not possible to demonstrate any clear field relationship between the distribution of high-pressure silica polymorphs and the extent of Lilly's metamorphic zone. On theoretical grounds, I agree that metamorphism must account at least in part for the absence of these minerals, but a much more detailed investigation is needed to clarify this aspect. Other factors may be also responsible for the absence of coesite and stishovite.

The post-shock metamorphism described by Lilly results probably from the high temperature of the rocks immediately before impact. Due to the great magnitude of this event, deep portions of the continental crust must have been shattered, at depths where temperatures attained several hundred degrees. In the Vredefort Dome these deep portions are represented by the lower part of the Witwatersrand Supergroup and by the granitic core, that is those rocks in which the post-shock metamorphism is developed.

After the quick uplift which occurred immediately after impact, rocks brought very close to the surface cooled down rapidly. This part is now eroded. In contrast, rocks which remained at a depth of several hundred metres or more, which presumably constitute the present-day outcrops, remained warm long enough to allow for the recrystallisation of shock metamorphism textures and reversal of coesite and stishovite into quartz. This probably explains the post-shock metamorphism, which in fact has been initiated before the impact.

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Expected shapes of asteroids

FUJIWARA *et al.*[1] have demonstrated experimentally that random breakage of basalt blocks to simulate asteroid formation produces a predominance of what the sedimentary petrologists call blade-shaped fragments; rod-like and sphere-like fragments tended not to be produced. These results agree well with a theoretical analysis[2] of the random breakage situation in which very simple probabilistic methods were used to estimate the formation of the four basic Zingg shapes (disks, spheres, blades and rods). It was proposed that, with an attainable 10% accuracy of dimensional resolution, random breakage of an isotropic rock material would yield 72% blade shapes, 27% tetragonal shapes (rods and disks) and 1% equiaxed shapes (spheres). The sphere value agrees well with the Fujiwara *et al.* results but the theoretical estimation did not allow rods and disks to be separated. It is possible that the experiments[1] produced several disk particles which were judged to be blades. In some earlier experiments Drake[3] examined various rock types; basalts and other aphanitic rocks produced approximately the same number of spheres as predicted by the theory but the number of blades was greater, at the expense of rods and disks; as Fujiwara *et al.* have also found.

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reviews

Major environmental controversy

B. A. Thrush

The Ozone War. By L. Dotto and H. Schiff. Pp. 342. (Doubleday: New York, 1978.) $10.

THE most interesting environmental controversy in recent years has surrounded the potential effects of supersonic aircraft and of chlorofluoromethanes (CFMs) on the stratospheric ozone which protects the Earth's surface from harmful ultraviolet light. Although the basic mechanism by which oxygen is converted into ozone had been propounded in 1930 by Chapman, the atmosphere had become so much a preserve of meteorologists and atmospheric physicists that, forty years later when it was suggested that the nitrogen oxides in the exhausts from supersonic aircraft would deplete the ozone layer, no-one knew what the natural amounts of nitrogen oxides in the ozone layer were.

In such circumstances, it is hardly surprising that projected fleets of 500 supersonic planes were predicted to produce large depletions of ozone, and that the major Climatic Impact Assessment Program (CIAP) was established in the United States. When CIAP's report appeared in 1975, the problem had largely disappeared because of the paucity of supersonic aircraft and better knowledge of the problem. By now, Molina and Rowland's paper (*Nature*, **249,** 810; 1974) had switched attention to the CFMs, particularly F-11 ($CFCl_3$) and F-12 (CF_2Cl_2) which are extensively used as aerosol propellants, refrigerants and foam-blowing agents. The great stability of these compounds ensures that they are eventually transported up to the stratosphere where their decomposition products will remove ozone. Because the predicted ozone depletions increase slowly to a maximum over a period of 50–100 yr, they would be very hard to detect against the short-term fluctuations in ozone cover; the argument therefore revolved around modelling calculations. To the thriving and vocal industries involved, 'theory' and 'hypothesis' rapidly became pejorative words, although research was soon financed. US Government agencies battled briefly for the 'lead' role. Many scientists joined the bandwagon and the Congressional Record sometimes provided rapid publication for unrefereed work. The public were often confused by contradictory news stories, and (although it is customary to blame the press for seeking scare stories) some scientists provided them willingly, others unwittingly through the difficulty of explaining a complex problem clearly.

This book gives a fascinating insight into the people and organisations who became involved in these controversies. To those accustomed to the more phlegmatic debate of such issues in this country *The Ozone War* may sound an exaggerated title, but many of the participants saw themselves as crusaders against the lack of action or the overreaction by Environmentalists, Industry or Government Agencies, with Congressional Hearings providing a natural sounding board.

The debate calmed down when the National Academy of Sciences (for once not described as 'prestigious' in a North American publication) appointed a panel to examine the CFM problem. Their report in 1976 concluded that release of F-11 and F-12 at the 1973 rates would eventually produce an ozone depletion of 7% with uncertainty limits from 2–20%. Predictably, some saw the upper limit as a cause for immediate action and others saw the lower limit as a reason for delaying action. Surprisingly, the latter have taken heart from recent discoveries which have reduced estimates of ozone depletion by supersonic aircraft and by increased use of nitrogenous fertilizers, despite the concomitant increases in the predicted effects of CFMs.

Surprisingly, there has been no debate on what would be an acceptable ozone depletion, and the potential effects of ozone depletion have received much less attention. A 10% decrease in ozone would increase damaging ultraviolet light by about 20%, probably causing a similar increase in skin cancer. However, such diseases are only responsible for about 1% of cancer deaths, although the figure is increasing rapidly because of the fashion for suntans in recent years. Is excessive sunbathing a health hazard or should we be more concerned about the considerable effect of increased ultraviolet light on phytoplankton? Will ozone depletion affect atmospheric circulation and therefore the climate? Here the answer is that it is probably less than that due to the increased carbon dioxide from burning fossil fuels.

Basically, the aerosol spray provides an easy target for the environmentalist, whereas the other uses of F-11 and F-12 in air conditioners, in insulating foam and in refrigerators require a more careful cost–benefit analysis. Another interesting point to be considered is how far different nations can agree about what is a global problem, as the effects of these compounds are quite independent of where on the Earth's surface they are released.

The history of how the vital processes in atmospheric chemistry were discovered and the rush to join the bandwagon are reminiscent of *The Double Helix* but with a much larger cast and broader setting in which Industry, Government Agencies and individual scientists strive to protect their own interests. The principal events and characters are well described and although one protagonist may not be pleased to see the first measurement of the rate of an important reaction attributed to another, the authors are to be congratulated on producing a highly readable and accurate account of how a major environmental controversy evolved in the United States. There are lessons here for all, particularly for those who wish to know how newspapers can come to publish contradictory or dogmatic reports about an unresolved issue. □

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Plant-parasitic nematodes

Ecology of Plant-Parasitic Nematodes. By D. C. Norton. Pp. 268. (Wiley: New York and Chichester, UK, 1978.) £16.95.

THIS book is an introductory account of plant-parasitic nematodes in relation to their environment. It is not an updated version of Wallace's book *Nematode Ecology and Plant Disease,* as the author tackles the subject from, what he terms, a broad ecological viewpoint. Emphasis is placed on the effects of the environment rather than on the disease aspect, and discussions on nematode damage to plants are largely omitted.

The first five chapters cover aspects

of nematode distribution, dispersal, habitats, groupings and communities, and population change. These include some interesting, but all too brief, discussions on the origins and recent past of nematodes, clustering, their means of dissemination, and some methods of analysing nematode communities and populations. It is well accepted that parasitic nematodes are always found associated with all forms of plant life; and, under the heading of habitats, the author mentions their associations with fungi, algae, lichens, mosses and higher plants. An introduction to population changes is presented in which seasonal changes, growth patterns, r and k selection, and sex ratios are discussed.

In the following two chapters the author deals with the soil environment: in the first of these, he discusses basic soil principles referring frequently to the interrelationships between soil factors; and in the second, he discusses the effects of physical and chemical soil changes on the nematodes, the section on effects of soil fertility is of particular interest to plant nematologists. The remainder of the book is concerned with survival, biological control, and interactions between nematodes and with other organisms. The final chapter is a disappointingly short account of the effects of agricultural practices on nematode populations, although the author acknowledges that the influence of cultural practices on nematodes has not received the attention it deserves.

There are errors throughout the book which could have been avoided—for example, the use of some defunct nematode nomenclature. Dr Norton has summarised much of the relevant literature in tabular form rather than discussing it in the text; even so these lists are not exhaustive. Most of the literature cited and the majority of the discussions relate to the ecology of nematodes in the United States. Although these are often relevant to work elsewhere, the book seems to have been written with the North American market in mind.

It is a useful introductory book to plant nematode ecology in which the author has brought together information from ecological disciplines relating to nematodes under one cover. Some interesting ideas are introduced, but it is not a sufficiently in-depth, critical or comprehensive study of the subject to entirely satisfy the needs of the professional nematologist. **John Bridge**

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Search for psychological reality

Linguistic Theory and Psychological Reality. By M. Halle, J. Bresnan and G. A. Miller. Pp.329. (MIT Press: Cambridge, Massachusetts, and London, 1978.) £12.25; $17.50.

THE search for psychological reality is on again. Last time the psychologists did the searching with the new toys they did not understand; this time it is the linguists (at least in approach) who are doing the searching and get the reviewer very confused in the process. It is a book without Chomsky, but with his bust on the mantlepiece, in which we start with the acceptance that linguistics is a branch of psychology but come away with the feeling that they mean that psychology is a branch of linguistics. Linguists and psychologists talk together; they write chapters which refer to each other (good editorial work here) but to scarcely anyone else.

We start with a chapter by Bresnan on "A realistic transformational grammar". The largest part of this is devoted to a beautiful linguistic demonstration of how some facts which used to be handled by transformations are now handled in the lexicon. The linguistics is generally judged among the best being produced currently and is very convincing. The 'realistic' in the title is introduced early. She makes the explicit claim that if a grammar is "to represent the knowledge of the language user in any psychologically interesting way" then all the grammatical rules must be realisable in a psychological model. If this is a serious claim then one might think that the best strategy would be to focus first on the types of constraint that psychology might impose. Instead we get the impression that the psychology is an afterthought. A hand reaches out languidly to pluck out a single piece of supporting data or intuition but no more. Thus Bresnan gives us reasons for arriving at passive and short passive forms directly instead of by means of transformations. At the end she notes that one of the other contributors to the volume reports that short passives (for example, the dog was bitten) was acquired before full passives by children. The tone of voice is almost congratulatory—how sensible of the children to realise the correct derivation! Such use of psychological evidence permeates the book.

Wanner and Maratsos give an example of a different kind of model, an Augmented Transition Network (ATN). One of their aims is to parse sentences without recourse to transformations. Strangely, then, we are also told several times that these parsers "are not incompatible with a transformational model of language". Remembering that Bresnan still found transformations necessary, this is reassurring. However, if Brenan's grammar is psychologically realistic and the ATN's have psychological reality doesn't the latter have to be the realisation of the former?

The lexical side of language is no better handled except in a little piece on "The child as a word learner" by Susan Carey. In this she exposes the magnitude of the task of pairing thousands of words with their meanings in a few years. No answers, but a hint of the psychological questions. George Miller has a chapter on the semantic relations among words. This is a development of the ideas he put forward with Johnson-Laird in *Language and Perception* (reviewed in *Nature;* **264,** 124, 1976). Miller makes some tight formal points but his thinking doesn't make as close a contact with psychological issues as the earlier work. Still, this is better than Jackendorf who, like Miller, wants to claim that semantics must derive from a general theory of conceptual structures. This seems a saner view of universality than one which bases it exclusively on language, but Jackendorf effectively turns the relationship around by using linguistic analysis to derive the semantic system from which the cognitive universals should emerge. The effectiveness of his argument may be judged from a nonlinguistic parallel which he describes—"the ability of a dancer to create appropriate dances for given music . . . in such a way that there is substantial interpersonal agreement on how appropriate the translation is." There must be generalisations which mediate the "complex and subtle" expression "determined at least in part along lines laid down by the nature of the organism." (Q.E.D.).

This kind of nativism gets another airing by Halle in a discussion of phonology where a 1971 result of Eimas is cited in support of innate mechanisms of speech analysis. Half-a-dozen or more recent refutations of this view have apparently gone unremarked (see, for example a chapter by Mehler in *Psycholinguistics,* series 2, ed. Morton and Marshall; Elek, 1979). There is a lot of intelligence in this book overall and a review by a linguist might give a much more positive impression but the *and* in the title is disjunctive.

John Morton

John Morton is on the scientific staff at the MRC Applied Psychology Unit, Cambridge, UK.

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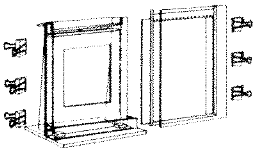

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Haematological overkill

The Year in Hematology, 1978. Edited by R. Silber, J. LoBue and A. S. Gordon. Pp. 517. (Plenum Medical: New York and London, 1978.)

ALL the signs are that we are entering a state of literary polycythaemia. For non-clinical readers this means that current scientific writing is suffering from a pathological excess of blood. As well as the increasing number of textbooks and monographs on the subject and a rapidly proliferating series of haematology journals, many of which carry review articles, there are regular review series such as *Clinics in . . .*, *Recent Advances in . . .*, *Topics in . . .*, *Seminars in . . .*, *Progress in . . .*, and many more, all offering minor variations on the same theme. Added to all this the general scientific and medical journals are giving a considerable amount of space to articles on haematological topics, and nearly every international meeting on the subject now insists that authors prepare manuscripts for a published account of the proceedings. Presumably somebody reads all these reviews and the publishing houses and the Inland (and Internal) Revenues (if not the authors) are waxing rich. However, as blood letting rather than transfusion is the more logical approach to the treatment of polycythaemia, surely the appearance of yet another review series in this field must raise serious questions about the value of all this repetitive writing.

The Year in Hematology first appeared in 1977 and on the jacket of the 1978 edition the series is described as offering "a comprehensive overview of the latest findings and trends that have occurred during the last year, critically appraising the most recent observations of normal and abnormal function in the hemopoietic system". A glance at the list of references at the end of each chapter and of their contents indicates that it does no such thing. In fact, this is yet another collection of review articles.

Because of this plethora of activity the editors of new haematological review series must exercise considerable ingenuity in finding topics which have not been covered many times in the previous year or two. In fact, there are one or two subjects which appear in this edition of *The Year in Hematology* which fall into this category, although not many. For example, the interesting chapters on the regulation of the egress of bone marrow cells, thymosin, and the effects of anti-cancer agents on haemopoietic progenitors deal with areas which have not been overexposed recently. On the other hand haemoglobin switching, cytogenetic studies in leukaemia, leukaemia antigens, and recent advances in the treatment of Hodgkin's disease and non-Hodgkin's lymphoma have all figured with monotonous regularity.

With a few exceptions the subjects covered in the 1978 *Year in Hematology* are dealt with thoroughly, referenced adequately and presented clearly. The book is well produced and illustrated. Perhaps it is unfair to judge this series after only two editions but the editors are certainly going to have to use all their imagination to really justify the existence of yet another review series in a field in which the number of 'overviews' seems to be in danger of exceeding the number of original articles which are available to be reviewed. Indeed, the horrid neologism 'overview' seems to be in real danger of taking on a rather different meaning to that which its inventors had in mind!

D. J. Weatherall

D. J. Weatherall is Nuffield Professor of Clinical Medicine at the University of Oxford, UK.

Green crop fractionation

Leaf Protein and Other Aspects of Fodder Fractionation. By N. W. Pirie. Pp. 183. (Cambridge University Press: Cambridge and London, 1978.) £8.

IMPROVED systems of crop production and utilisation attract world-wide attention. The technique of fractionation to produce high quality foods for humans and simultaneously feeds for farm livestock from fibrous green crops comes into this category. For approaching half a century N. W. Pirie has inspired, with his determination and enthusiasm, much research in both the developing and more developed regions of the world. Students, teachers, scientists and others with a devotion to improving food production, including those with particular interests in fractionation, would do well to read the book and to absorb the vast amount of investigation which has already taken place.

Leaf protein is potentially a most abundant source of protein with a good nutritive value, and temperate and tropical flora examined as sources of extractable protein are numerous. The separation and preparation of good fractionation products is well documented and these can be stored safely to prevent deterioration. A vast amount of qualitative information is already available from the scientific literature and the need now is for more quantitative data: crop and machines used must be thoroughly defined so that the end-products cease to be mysterious. The main challenge now is to assess when the exciting technical potential can be harnessed to provide worthwhile practical systems.

N. W. Pirie has always been interested in the less developed areas of the world and already there is evidence of sub-tropical villagers using leaf protein as food. The expansion of this type of work needs a missionary approach and substantial financial support to act as the catalyst. Adoption of the technique has been slow. At the present time a greater interest seems to exist for exploiting the fractionation technique on a factory scale to produce leaf protein more as a source of pigment for poultry rations than as a source of protein. Some units already exist in Europe and North America and a modest expansion of this use of the technique is likely to occur around the world. Whether as a 'village' or a 'factory' operation the prospects for fractionation need discussion and debate. Further research is also required and a number of proposals are made in the chapters of this book.

Greater attention should be directed to improving the efficiency of green crop production and utilisation. Although it is technically possible by fractionation to produce leaf protein, We must not lose sight of the fact that some 80% of the harvested dry matter is best suited for ruminant consumption and certainly cannot be discarded. In many ways the use of fibrous green crops for human foods is exciting but there is a necessary balance between this and ruminant feeding.

Two centuries after the death of Hilaire Marin Rouelle, who published the first scientific papers on leaf protein, the world is certainly no less dependent on the politicians and the economic climates they create and foster. Progress has been made in green crop fractionation and the prospects are tantalising ecpecially if scientists in the wake of N. W. Pirie can help the necessary biochemical engineering to take place and develop reliable systems robust enough to be profitable compared with alternatives irrespective of the scale and objectives of the operation. Investigation of the future prospects of green crop fractionation deserves support and the book is a timely publication.

J. Connell

J. Connell is Principal Scientific Officer at the National Institute for Research in Dairying, Shinfield, UK.

Gregory Rift Valley and early man

Geological Background to Fossil Man. Recent Research in the Gregory Rift Valley, East Africa. Edited by the late W. W. Bishop. (Scottish Academic Press: Edinburgh, 1978.) £25.

THE subtitle of this book is important, but it needs some explaining. The Gregory Rift Valley, for the benefit of non-geologists, is part of the great East African Rift—the system of valleys bounded by faults that extends southwards from the Red Sea for a distance of some 3,000 km. The section of this system known as the Gregory Rift, if one is to be precise, runs only for about 450 km from the bottom end of Lake Turkana in northern Kenya to the Lake Natron and Ngorongoro areas in northern Tanzania. But for the purposes of this book, the Gregory Rift has been interpreted more widely to include the northwards extension of the Rift—otherwise called the Ethiopian Rift—to the Afar in northern Ethiopia. The important early man sites at Hadar, and those in the Omo Valley and east of Lake Turkana thus come within the book's scope.

By geological accident, the Gregory Rift Valley—in both narrow and wide senses—is a field laboratory perhaps without equal for palaeontological and archaeological studies of early man as well as for investigating the structural, volcanic and geophysical aspects of rift systems. It was therefore a good idea of the late W. W. (Bill) Bishop's to organise in February 1975 a meeting at the Geological Society of London of many of the geologists and others who had worked or were still working in different parts of this great valley. This book—a collection of 35 papers—is the outcome. Not all the contributions are geological, but all in one way or another add to knowledge of the development of the Gregory Rift Valley and the environment of early man at different times in this part of East Africa.

A major part of the book is about the geology and palaeontology of the Baringo Basin. This was the area in which Bishop, and also Basil C. King—Director of the East African Geological Research Unit and Bishop's Professor for a period—devoted much of their own and their students' time between the mid-1960s and mid-1970s. It is a loss to science that with Bishop's tragically early death in 1977 and King's retirement from the chair of geology at Bedford Colege, London, systematic geological and palaeontological surveying in the Baringo area has almost ceased, although an archaeological project at Chesowanja does continue.

As events have turned out, the book has become a memorial tribute to Bishop's geological work in East Africa. It is therefore a pity that the volume is not all that it might have been. Bishop unfortunately died before he had completed the introduction to the three main parts and also the summing-up, so the book lacks these sections—though this is not the publisher's fault. I was, however, sorry to see so many typographical errors and minor editorial inconsistencies for which the publisher should take responsibility. These are understandable in the circumstances, but are to be regretted nonetheless. I should also mention that 'recent' in the subtitle means no later than 1975–76.

Geological Background to Fossil Man remains, however, a useful record of research in the Gregory Rift Valley and is especially valuable for the many geological maps and sections it contains. A special feature, and one that is rare these days, is the inclusion of a 1 : 10,000 coloured, foldout geological map of the area around Olorgesailie Prehistoric Site in Kenya. This map was prepared in 1946 by Robert M. Shackleton for the First Panafrican Congress of Prehistory in Nairobi but money was not available then for its publication. For this edition, grants towards the cost of publication have come from the Royal Society and the L. S. B. Leakey Foundation. The names of the stratigraphical units have been updated and the map was specially redrawn by Barbara Isaac. It accompanies a detailed account by Glynn Ll. Isaac of the geology of the Olorgesailie Formation. **Sarah Bunney**

Sarah Bunney was recently Editor at The International Louis Leakey Memorial Institute for African Prehistory in Nairobi, Kenya.

Ion-selective electrodes

Analysis with Ion-Selective Electrodes. By J. Vesely, D. Weiss and K. Stulik. Pp.243. (Wiley: New York, London and Sydney, 1978.) £16.

THE Ellis Horwood Series in Analytical Chemistry under the guidance of Dr R. A. Chalmers (Editor of Talanta) and Dr Mary Masson, of the University of Aberdeen, will be useful both to professional analysts, working in industry and the public services, and those few who teach analytical chemistry as a specialist subject in universities and polytechnics.

This volume is concerned with the applications of ion-selective electrodes as "sensors" that instrument technologists will need to interface with microprocessors—for example, when monitoring sodium and potassium levels for hospital patients. The authors are from Prague, home of J. Heyrosky and polarography fame, which guarantees that their work rests on a good theoretical background. The translation is smooth and clear, so that English readers can easily follow their text.

The work is divided into four chapters; the last is long and devoted to analytical applications. The first two deal with introductory theory and instrumentation, in some seventy pages with more than 300 references. Exact treatments are given of the theory of membrane potentials and activities, classification of electrode types with details of their selectivities and response characteristics, and information about measuring instruments used in potentiometry. The third chapter gives the experimental techniques used, including directions for the preparation and presentation of samples for analysis, descriptions of discrete and continuous measurements with explanations of calibration and standardisation techniques. This chapter finishes with an account of automated methods of analysis and the use of ion-selective electrodes in biochemistry, biology and medicine.

The final long chapter surveys the applications, as reported in the world's literature until about the end of 1977. It seems to be comprehensive; some 840 references are reviewed in 78 pages. These are dealt with rather in order of the reliability of present-day electrodes, starting with the applications for halogens and cyanide in greatest detail and continuing with sulphur compounds, nitrogen (including enzyme reactions), phosphorous and other nonmetals more briefly. Cation-selective electrodes are treated in the same way, starting with alkali and alkaline earth metals, followed by silver, mercury and so on. The book continues with tabulations for the determination of various inorganic materials and organic compounds and concludes with an Appendix of useful data tables.

This then is a valuable text and one does not need to criticise such honest work on the part of the authors. However, it is hoped that at a later time they may be encouraged to include the glass pH electrode, purposely avoided in this volume, as it is still the 'best' ion-selective electrode.

J. A. W. Dalziel

J. A. W. Dalziel is Reader in Analytical Chemistry at Chelsea College, University of London, UK.

announcements

Meetings

4–18 March, **Comparative Biology of the Nervous System,** Caracus (Dr Guillermo Whitfembury, Director of CLAB, Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracus, Venezuela).

9–10 March, **New York Einstein Centennial Celebration** (Einstein Committee, The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021).

12–16 March, **Labex International '79,** Birmingham (Industrial and Trade Fairs Ltd, Radcliffe House, Blenheim Court, Solihull, West Midlands, UK).

13–16 March, **Tunnelling '79,** London (Christopher Bradley Ltd, 78 Vineyard Hill Rd, Wimbledon Park, London SW1, UK).

15 March, **Charge Coupled Devices in Signal Processing,** London (Dr A. Holmes-Seidle, Fulmer Research Institute, Stoke Poges, Slough, UK).

27–29 March, **SEA-CON (79),** Brighton (C. G. Hague, Kent Instruments Ltd, 27B Bell St, Reigate, Surrey, UK).

28–30 March, **Process Engineering—the Future,** Loughborough (The Conference Secretariat, Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

1 April, **More Physics, Less Relevance?,** Cambridge (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

3–5 April, **Annual Chemical Congress,** Bristol (Dr John F. Gibson, The Chemical Society, Burlington House, London W1, UK).

3–6 April, **3rd International Symposium on Distillation,** London (S. A. E. Buxton, The Institution of Chemical Engineers, 165-171 Railway Terrace, Rugby, UK).

9–11 April, **River Pollution Control,** Oxford (Conference Organiser, Water Research Centre, Medmenham Laboratory, Henley Rd, Medmenham, Bucks, UK).

9–11 April, **Enzyme Inhibitors as Drugs,** London (Mrs J. Kruger, Dept of Pharmacology, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1, UK).

9–11 April, **Pollution from Road Vehicles,** Warwick (NSCA, 136 North St, Brighton, Sussex, UK).

10–11 April, **Symposium on Straw Decay,** Hatfield (Dr E. Grossbard, Hatfield Polytechnic, Bayfordbury House, Lower Hatfield Rd, Hertford, UK).

16–21 April, **Freeze-etching in Electron Microscopy,** Woods Hole (Admissions Office, MBL, Woods Hole, Massachusetts 02543).

18–19 April, **Photochemistry, Lasers and Spectroscopy,** Newcastle upon Tyne (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V0, BN, UK).

2–4 May, **Drug Treatment in Chronic Cerebrovascylar Disorders,** Milan (Dr G. de Gaetano, Istituto di Ricerche Farmacologiche "Maro Negri", 20157 Milano, Via Eritrae, 62 Italy).

8 May, **Efficient Use of Resources: Implications for the Agricultural Engineer,** Kenilworth (Mrs E. J. Holden, The Institution of Agricultural Engineers, West End Rd, Silsoe, Bedford, UK).

8 May, **International Symposium on Crop Protection,** Gent (Rijksuniversiteit Gent, Faculteit van de Landbouwwetenschappen, B-9000 Gent, Belgium).

9–11 May, **Water Research Centre Open Days,** Medmenham (WRC PO Box 16, Henley Rd, Medmenham, Marlow, Bucks, UK).

9–11 May, **From Molecules to Community: Multifaceted Mental Health Research,** Saskatoon (D. G. Irvine, Psychiatric Research Division, University Hospital, Saskatoon, Saskatchewan, Canada S7N 0W8).

27 May–1 June, **14th World Gas Conference,** Toronto (Conference Office, PO Box 1979, Commerce Court West, Toronto, Ontario, Canada M5L 1J4).

6–7 June, **Mechanisms of Viral Pathogenesis and Virulence,** Munich (P. A. Bachmann, WHO Collaborating Centre, Koniginstr. 49, 8000 Munich 22, FRG).

17–19 June, **21st National Annual Congress of the Argentine Neurosurgical Association,** Santa Fé (Dr C. Cortelezzi, Casilla de Correo 1936, Buenos Aires, Argentina).

18–20 June, **Processes and Water Quality Modelling,** New Orleans (Dr P. Hamilton, Science Applications Inc., 4900 Water's Edge Drive, Suite 255 Raleigh, NC 27606).

18–21 June, **Metabolic Effects of Alcohol,** Milan (Organising Secretariat, Centro Studi de' Alimentazione, Nutrition Foundation of Italy, Via S. Pietro Allberto 17, 20121 Milan, Italy).

25–29 June, **Excess Electrons and Metal—Ammonia Solutions,** Aviemore (Dr B. C. Webster, Chemistry Dept, The University of Glasgow, UK).

Reports & publications

UK & Ireland—December

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 284, No. 1002: The Biochemical Functions of Terpenoids in Plants. A Discussion organised by T. W. Goodwin, FRS. Pp. 437–603+3 plates. (London: The Royal Society, 1978.) UK £10.90; Overseas £11.40. [412

Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research. Fisheries Radiobiological Laboratory. Technical Report FRL 14: Radioactivity in Surface and Coastal Waters of the British Isles, 1976. Part 2: Areas Other Than the Irish Sea and Its Environs. By N. T. Mitchell. Pp. 20. (Lowestoft: Ministry of Agriculture, Fisheries and Food, Fisheries Radiobiological Laboratory, 1978.) [412

Loughborough University of Technology: Department of Civil Engineering. Water and Waste Engineering for Developing Countries. 4th WEDC Conference: Engineering for Health in Hot Countries, 25th–27th September 1977. Edited by John Pickford. Pp. 192. (Loughborough, Leicestershire: Mrs Rowena Steele, Department of Civil Engineering, University of Technology, 1978.) Softback £8; Hardback £12. [412

Third Report from the Select Committee on Science and Technology, Session 1977–78. The Durability and Efficiency of Filament and Discharge Lamps. Vol. 1: Report. Pp. xxxiii. 80p net. Vol. 3: Appendices to the Minutes of Evidence. Pp. vii+267–452. £3.10 net. (London: HMSO, 1978.) [512

The Edinburgh School of Agriculture. Annual Report, 1977. Pp. 239. (Edinburgh: The Edinburgh School of Agriculture, West Mains Road, 1978.) [612

Home Office. Report of the Committee on Data Protection. Chairman: Sir Norman Lindop. Pp. xxiv+460. (Cmnd. 7341.) London: HMSO, 1978.) £6 net.

A Review of Auxiliary and Booster Fan Ventilation Practice in Mines. (A Report by the National Committee to Examine All Aspects of the Ventilation of Narrow Drivages.) Pp. 41. (London: HMSO, 1978.) £2.25 net. [712

Department of the Environment; Scottish Office; Welsh Office. Annual Survey of Radioactive Discharges in Great Britain, 1977. Pp. 42. (London: Department of the Environment, Waste Management Division, Becket House, Lambeth Palace Road, 1978.) 70p. net. [712

Department of Industry. Report of the National Committee on Computer Networks. Presented to the Secretary of State for Industry by J. Howlett, CBE. Pp. vi+121. (London: Department of Industry, Dean Bradley House, 52 Horseferry Road, SW1, 1978.) *gratis.* [712

Annual Report of the British Council, 1977–78. Pp. 72. (London: The British Council, 10 Spring Gardens, SW1, 1978.) [712

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APPOINTMENTS VACANT

UNIVERSITY OF HONG KONG
DEPARTMENT OF ANATOMY
SENIOR LECTURESHIP IN ANATOMY
(Re-advertisement)

Following the establishment of a Dental School, applications are invited for appointment from September 1, 1979, to a post of Senior Lecturer in Anatomy.

Applicants should be either dental, medical or science graduates with experience in teaching anatomy to preclinical dental students. The appointment carries the responsibility for coordinating and implementing all anatomical teaching within the dental course. The appointee will be expected to engage in research work and may be required to teach other students on occasion. Facilities exist within the Department for a major research interest to be built up in one of the oral biology fields.

Annual salary (superannuable) is: HK$98,700 by 4,260 to 120,000 by 4,200 to 132,600 (£1=HK$9.40 approx.). Starting salary will depend on qualifications and experience.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or the Assistant Secretary (Recruitment), University of Hong Kong, Hong Kong.

Closing date for applications is **March 15, 1979.** 9569(A)

Central Birmingham Health District
BIRMINGHAM MATERNITY HOSPITAL
Infant Development Unit
CYTOGENETICIST
(Principal Biochemist Grade)
(£6,999 to £9,177)

Applications are invited for this new ost of Principal Cytogeneticist. The aboratory offers a full range of cytoenetic investigation and has Regional s well as District commitments. The ppointee will be responsible to the Consultant Pathologist for the organiation of work within the laboratory, uality control and the introduction of ew techniques. Applicants should old a relevant science degree and be ully experienced in the field of Cytoenetics.

Visits to the laboratory may be rranged by appointment with Dr ameron, Tel. 021 454 4851 ext. 224 Children's Hospital).

Application forms and job description from Personnel Officer, Birmingam Maternity Hospital, Birmingham 15 2TG. Tel. 021 472 1377.

9511(A)

UNIVERSITY OF MALAWI
Bunda College of Agriculture

Applications are invited for the following posts in the

CROP PRODUCTION DEPARTMENT

tenable from September 1, 1979:

LECTURER IN BIOMETRY

Applicants should have a good first degree in Agriculture, Mathematics, or the natural sciences with postgraduate qualifications and experience in statistics as applied to agriculture. The appointee will be responsible for teaching agricultural statistics.

LECTURER IN HORTICULTURE

Applicants should have a good first degree in Agriculture or Horticulture with postgraduate qualifications in Horticulture. Practical research or production experience in Horticulture would be an advantage. Duties of the post include teaching the horticulture aspects of crop production and establishing a research programme in fruit or vegetable production.

LECTURER IN SOIL SCIENCE (Two Posts)

Applicants should have a good first degree in Agriculture or Soil Science with postgraduate qualifications and practical field experience in soil chemistry or soil physics. Duties of both posts include teaching the soil environmental aspects of crop production.

LECTURER IN AGRICULTURAL ENTOMOLOGY

Applicants should have a good degree in Agriculture, Biology or Zoology with postgraduate qualifications and experience in entomology as applied to agriculture. Duties of the post include teaching entomology as part of the crop protection element of crop production.

Salary Scale: K3,500 to K6,600 p.a. (£1 sterling=K1.62.) The British Government may supplement salaries in range £3,450 to £3,654 p.a. (sterling) for married appointee and £2,340 to £2,544 p.a. (sterling) for single appointee (reviewed annually and normally free from all tax) and provide children's education allowances and holiday visit passages. If no British Government supplementation is available, the University may pay an addition of between K1,320 to K1,656 p.a. (taxable in Malawi). Family passages, superannuation scheme; gratuity 15 to 25%; biennial overseas leave; housing and various allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar, University of Malawi, P.O. Box 278, Zomba, Malawi, by March 19, 1979.

Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9568(A)

MEDICAL RESEARCH COUNCIL
VIROLOGY UNIT
Institute of Virology
UNIVERSITY OF GLASGOW

A vacancy exists for a research scientist with experience in enzymology and/or protein biochemistry. The successful candidate would investigate the properties of certain Herpes Simplex Virus induced biologically active polypeptides (e.g. DNA polymerase, thymidine kinase), and be expected to pursue individual research problems as well as collaborative projects with other staff. Previous experitnce with animal virus systems is advantageous, but not essential.

The position is a three or five year limited-term appointment on the M.R.C. non-clinical scientific staff with salary starting at an appropriate point on the Grade II scale (£3,883 to £6,080). M.R.C. Pension Scheme. Applicants should send a curriculum vitae giving full details of qualifications and experience and the names of three Referees to:-

Professor J. H. Subak-Sharpe,
Institute of Virology,
University of Glasgow,
Church Street,
GLASGOW,
G11 5JR

by **February 28, 1979.** 9486(A)

UNIVERSITY OF BRISTOL
DEPARTMENT OF PATHOLOGY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from biological scientists with interests in virology, cell biology, or immunology. The successful applicant will join a team investigating lymphocyte-virus interactions during EB virus infection in vitro.

This grant-supported appointment will carry a salary on the scale beginning £3,833 per annum, depending on experience. (Scale under review.) The appointment will be for two years in the first instance, and is likely to be renewed thereafter for a further three years.

Replies in writing, giving the names and addresses of two referees to Professor M. A. Epstein, Department of Pathology, The Medical School, University Walk, Bristol BS8 1TD, by February 28, 1979. 9580(A)

Southampton THE UNIVERSITY
DEPARTMENT OF PHYSICS

Applications are invited for two **LECTURESHIPS** in the Department of Physics. The persons appointed will be expected to play a full part in the Department's programme of teaching and research in experimental physics. It is likely that preference will be given to candidates with research experience in the fields of condensed matter physics, low-temperature physics and space physics.

Salary scale £3,883 to £7,754 per annum plus U.S.S. The initial salary will depend on qualifications and experience.

Further particulars may be obtained from Mrs E. C. P. Sears, Staffing Section, The University, Southampton SO9 5NH to whom applications (7 copies from United Kingdom applicants) should be sent not later than March 26, 1979. Please quote reference number 1703/A/Na. 9470(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
RESEARCH ASSISTANT IN BIOCHEMISTRY

urgently required to study the biological role of peptides released after activation of pancreatic zymogens. A knowledge of peptide chemistry and/or organ culture desirable but not essential. The appointment is in the Department of Surgery and will be for three years in the first instance with the possibility of registering for a Ph.D. degree.

Emoluments according to experience; minimum starting £3,834 p.a. inc. London weighting.

Please send curriculum vitae to Dr D. Grant, Department of Surgery, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. There is no closing date, suitable applicants will be interviewed immediately. 9593(A)

oxford polytechnic
DEPARTMENT OF BIOLOGY
TECHNICIAN IN HUMAN PHYSIOLOGY

(Salary £3,732 to £4,146)

Candidates should have degree, HNC, or equivalent qualification, and experience with electrophysiological equipment. Placement on salary scale according to age and experience.

Further details and application forms may be obtained from **the Deputy Administrator, Oxford Polytechnic, Oxford OX3 0BP.** 9563(A)

UNIVERSITY OF JOS
JOS NIGERIA

VACANCIES IN THE FACULTY OF MEDICAL SCIENCES

Applications are invited from suitably qualified candidates to fill the following vacancies in the Faculty of Medical Sciences of the University of Jos.

A. *POSTS*:

Professor/Reader/Senior Lecturer/Lecturer I and II in the following specialities: —

(1) Medicine
(2) Paediatrics
(3) Pharmacology and Therapeutics
(4) Psychiatry
(5) Surgery
(6) Obstetrics and Gynaecology
(7) Community Medicine
(8) Anaesthesia
(9) Microbiology
(10) Chemical Pathology
(11) Pathology
(12) Haematology and Blood Transfusion.

(i) *Professor/Reader*

Qualifications:

The Candidate, who should have a post-graduate qualification registerable with the Nigeria Medical Council, should be an authority in his field and should have several years' experience in his area of competence. He should have evidence of undoubted ability in research, such as contributions in reputable journals and should be capable of directing and supervising the research of junior colleagues. He should be capable of giving both administrative and academic leadership.

(ii) *Duties*:

The successful candidate would be involved with planning and organising undergraduate clinical programmes and in research. He would be expected to have the resourcefulness and energy required to meet the challenge of a new department.

B. *Senior Lecturer/Lecturer*:

(i) *Qualifications*:

Candidates for 1, 2,, 3 and 4 should possess an M.R.C.P. or similar post-graduate qualification.
Candidates for 5, should possess an F.R.C.S.
Candidates for 6, should possess an M.R.C.O.G.
Candidates for 7, should possess D.P.H./M.P.H. and possession of an M.R.C.P. or M.F.C.M. would be an advantage.
Candidates for 8, should possess an F.F.A.R.C.S. or D.A.
Candidates for 9, 10, 11 and 12 should possess an M.R.C. Path., a Ph.D. or equivalent qualification.

In the case of Senior Lecturers, candidates should possess not less than five years' experience in research and teaching in the speciality. Possession of a Ph.D. would be an advantage, as would training and experience in any sub-speciality in these fields.

(ii) *Duties*:

Successful candidates would be expected to be capable of interdisciplinary research and teaching, and would be responsible to the Head of Department for the organisation of clinical instruction.

C. *SALARY*:

Professor ...	... GL 16 — N11,268 x 576 — N12,420 per annum
Reader ...	... GL 14 — N 8,868 x 320 — N 9,828 per annum
Senior Lecturer	... GL 13 — N 7,764 x 320 — N 8,724 per annum
Lecturer I	... GL 11 — N 6,444 x 180 — N 6,984 per annum
Lecturer II	... GL 10 — N 5,460 x 162 — N 6,432 per annum

(N 1 equals approx. £0.80)

D. *CONDITIONS OF SERVICE*:

Successful candidates will be on probation for the first two years and afterwards confirmed to retiring age if their services are considered satisfactory. Other fringe benefits include pension scheme, car allowance, part furnished accommodation, or housing allowance in lieu of accommodation. 25 per cent Contract Addition is payable to all expatriates.

E. *METHOD OF APPLICATION*:

(a) Interested candidates in Nigeria are required to forward detailed applications (ten copies) to the Registrar, University of Jos, P.M.B. 2084, Jos, giving the following information: Post desired, full name, date and place of birth, nationality, permanent address, telephone number, if any, current postal address and telephone number, if applicable, marital status, number and ages of children, educational institutions attended with dates, full qualifications plus copies of certificates, previous employment with dates, present employment with dates and salary, list of publications, names and addresses of three distinguished scholars in relevant field as referees, two passport photographs of self. Candidates are advised to request their referees to forward references on them directly and under confidential cover to the Registrar to reach him not later than February 14, 1979.

(b) Overseas candidates should forward their applications (four copies) to the Principal Assistant Secretary (Recruitment), National Universities Commission, London Office, 180 Tottenham Court Road, London W1P 9LE, England, marked "Registrar/Jos" and giving the details listed in (a) above. Similarly, candidates should request their referees to forward references on them directly and under confidential cover to reach the London Office by February 14, 1979.

N.B. Further details are available from the London Office.

YSD/SA: REGISTRAR.

9574(A)

IMMUNOLOGIST/ VIROLOGIST

Two positions are available immediately at the Assistant Professor level for a Cellular Immunologist and a Virologist to work in the Bovine Leukemia Research Unit. Applicants should have a Ph.D. or D.V.M. degree with post-graduate training in the appropriate discipline.

Qualified applicants should send a résumé, copies of representative publications and the names of three people who may be contacted for references to:, Dr Jorge F. Ferrer, Chief, Section of Viral Oncology, University of Pennsylvania School of Veterinary Medicine, New Bolton Center, R.D. 1, Kennett Square, Pa. 19348. 9298(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
RESEARCH ASSISTANT IN CELL BIOLOGY

required to join a Cancer Research Campaign funded 3-year project investigating human pancreatic and other common cancers. Experience in cellular assays and tissue culture desirable but not essential.

Emoluments £3,834 to £4,333 (under review). The successful candidate will be expected to obtain a Ph.D. degree.

Applications and further particulars from the Establishment Officer, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. 9586(A)

CHILDREN'S HOSPITAL BIRMINGHAM
TECHNICIAN OR BIOLOGICAL SCIENCE GRADUATE

required for a research post. The successful applicant will be concerned with a leukaemia and non-Hodgkins Lymphoma study. Previous experience in immunology helpful but not essential. Appointment for one year in the first instance.

Salary according to experience and qualifications.

For further details and an application form please contact Dr F. G. H. Hill, Consultant Haematologist, Haematology Department, The Children's Hospital, Ladywood Middleway, Birmingham B16 8ET. Telephone: 021-454 4851. Closing date: March 15, 1979. 9590(A)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF GEOLOGICAL SCIENCES
LECTURER IN HYDROGEOLOGY

Applications are invited for the above post, the duties of which will principally include the teaching of Hydrogeology at M.Sc. Course level and the supervision of Course project work. The successful applicant will be expected to pursue research in the field of hydrogeology.

Applicants should have a good honours degree. A postgraduate qualification in Hydrogeology and practical experience would be advantageous.

Salary, according to age and experience, in the range £3,883 to £7,754 (to be reviewed) plus superannuation.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (3 copies) including curriculum vitae and naming three referees should be sent by March 2, 1979.

Please quote: Ref NG3. 9589(A)

UNIVERSITY OF LIVERPOOL
SCHOOL OF DENTAL SURGERY
TECHNICIAN

to work in electron microscope unit Research interests are concerned principally with mineralised tissues The unit is shortly to be equipped with a Jeol Temscan system with Link energy dispersive analytical equipment Previous experience in electron microscopy an advantage but training could be given to suitable applicant with previous relevant laboratory experience ONC or equivalent as minimum qualification.

Salary on scale up to £3,060 p.a. (under review) according to qualifications and experience.

Application forms may be obtained from The Registrar, The University, P.O. Box 147, Liverpool L69 3BX.

Quote Ref: RV/481/N. 9579(A)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF LINGUISTICS
RESEARCH ASSOCIATE

for S.S.R.C. Project on the Intelligibility of Speech to Children, for two years from March, 1979.

Qualification in psycholinguistics and/or linguistics plus some familiarity with experimental method required.

Salary: £3,883 to £4,382 per annum.

Applications with curriculum vitae and names of two referees to the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, by February 16, 1979.

Please quote: Reference 5004.

9577(A)

UNIVERSITY OF LEICESTER
PHYSICS RESEARCH Ph.D.

The Department of Physics offers excellent research opportunities leading to the degree of Doctor of Philosophy in the following areas:

IONOSPHERIC PHYSICS
NUCLEAR ELECTRONICS
QUANTUM MOLECULAR PHYSICS
SOFT X-RAY SPECTROSCOPY
SOLID STATE PHYSICS
THEORETICAL PHYSICS
X-RAY ASTRONOMY

Further details and application forms may be obtained from Professor J.L. Beeby, Department of Physics, University of Leicester, Leicester LE1 7RH. 9488(A)

UNIVERSITY OF EDINBURGH
THE EDINBURGH SCHOOL OF AGRICULTURE
LECTURER IN ANIMAL PRODUCTION

Applications are invited from suitably qualified graduates for a post of LECTURER IN ANIMAL PRODUCTION. The successful candidate will be involved in courses for postgraduate, undergraduate and HND students and will be encouraged to develop research interests.

Salary on scale £3,883 to £7,754 per annum.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, with whom applications should be lodged not later than March 9, 1979.

Please quote: Reference 1014.

9578(A)

CAREERS in SCIENTIFIC COMPUTING and ELECTRONIC ENGINEERING

CHALLENGING OPPORTUNITIES TO WORK ON:

- **MICROPROCESSORS**
- **REAL-TIME PROGRAMMING**
- **NETWORKS**
- **LOGIC DESIGN**
- **INSTRUMENTATION**
- **ACCELERATOR CONTROL SYSTEMS**

Daresbury Laboratory is developing major national facilities in the fields of computer networking, atomic, molecular and biological sciences and nuclear physics. The exploitation of these facilities requires first-class computing support. Computers at the laboratory include an IBM 370/165, GEC 4070s, PDP-11s and Interdatas.

• COMPUTER NETWORKS:
Development and support of communications software for interactive terminal, graphics and remote job entry access to the SRC's computing centres using microprocessor based communications hardware and mini-computer pocket switches and front end processors.

• LOGIC DESIGN:
Interface design for mini-computers using the latest microprocessor techniques, also communications interfacing for remote data processing. Specialised design of data acquisition systems.

• DATA ACQUISITION:
Programmers are required for the development of data acquisition systems using LSI and PDP-11 computers connected to an IBM 370/165. There are opportunities to work with large and small systems in a stimulating scientific environment. Relevant programming experience would be an advantage but is not essential.

• INSTRUMENTATION:
Design and development of specialised analogue and digital instrumentation for processing electrical signals from a wide range of charged particle and photon detectors. The most up-to-date facilities for R&D are available.

• ACCELERATOR CONTROL SYSTEMS:
Development of an on-line process control system for the Daresbury Synchrotron Radiation Source (a 2 GeV Electron Storage Ring), using Interdata computers. Experience in programming in both high level and assembler languages, and also in digital interface hardware, would be an advantage.

Applicants should have a good honours degree in a relevant scientific discipline. Appointments will be made depending on qualifications and experience at SO level.

Scientific Officer - £2,839-£4,415 (Salaries are currently under review)

Closing date: 15th March, 1979.

Please write or telephone: Warrington 65000, Ext. 467, for an application form quoting reference No. DL/65

The Personnel Officer
DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD.

9509(A)

UNITED KINGDOM ATOMIC ENERGY AUTHORITY

Energy..Energy.. Energy...

The potential of both conventional and unconventional energy technologies in helping meet future energy needs is a topical and important subject.

If the full potential of these technologies is to be realised, research, development and demonstration programmes all have a vital part to play.

The Energy Technology Support Units of the U.K.A.E.A., located at Harwell, is responsible on behalf of the Department of Energy for initiating and managing many of these programmes, all of which are now expanding.

SCIENCE OR ENGINEERING GRADUATES in their late twenties or early thirties, are needed in the following areas:-

-Planning the national programmes.
-Promotion and development of new and existing technologies in all areas of energy use to establish greater efficiency.
-Continuing development of solar, wind, wave, geothermal and tidal programmes.

Especially welcome are applications from those with industrial experience or those able to discuss technological matters effectively with senior people in both industry and Government.

Salary range: £5,514-£7,380

Write or 'phone: Steve Beackon, Personnel Department, UKAEA, Harwell, OXON, OX11 0RA. Abingdon (0235) 24141. Ext. 2058. 9507(A)

HARWELL

UNIVERSITY OF DUBLIN
Trinity College

LECTURER IN PURE MATHEMATICS

Applications are invited for the above post in School of Mathematics, Trinity College, Dublin, which will become vacant on October 1, 1979.

Salary scale: £4,232 to £8,321 p.a. Appointment is likely to be made within the range £4,232 to £4,680.

Application forms and further particulars relating to this post may be obtained from:

The Establishment Officer,
West Theatre,
Trinity College,
Dublin 2,

The closing date for receipt of applications will be **March 1, 1979.** 9487(A)

UNIVERSITY OF READING LECTURERSHIP IN PHYSIOLOGY AND BIOCHEMISTRY

Applications are invited for a Lectureship in Physiology. Candidates should have a degree in Physiology or related subjects, with interests in cardiovascular, respiratory, renal or muscle physiology and biochemistry and evidence of active research.

The appointment will be in the Lecturer salary scale but, other things being equal, within the range £3,883 to £5,842 per annum.

The person appointed should take up duties on June 1, 1979 or as soon as possible thereafter.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than March 10, 1979. 9534(A)

UNIVERSITY OF GLASGOW LECTURESHIP IN MATHEMATICS

Applications are invited for a Lectureship in the Department of Mathematics, duties to commence on October 1, 1979. Salary will be within the range £3,975 to £5,830 of the Lecturers' scale £3,975 to £8,250 according to qualifications and experience (which may be in any branch of Pure or Applied Mathematics).

Further particulars may be obtained from the Secretary of the University Court, (Room 18), University of Glasgow, Glasgow G12 8QQ, with whom applications (8 copies), giving the names and addresses of three referees, should be lodged on or before March 31, 1979.

In reply please quote Ref. No. 4362M. 9556(A)

ST. HELIER HOSPITAL
Carshalton, Surrey

POSTDOCTORAL RESEARCH ASSISTANT

required to work in Dermatology Department using immunological and biochemical techniques to study various aspects of skin disease including Cancer.

Salary according to experience.

Application, with full details of experience and names and addresses of three referees to Dr E. Rhodes, Dermatology Department at above address, from whom further details can be obtained. 9564(A)

UNIVERSITY OF KENT AT CANTERBURY
Appointments in Electronics

Applications are invited for the posts of

LECTURER AND TUTORIAL FELLOW
in Electronics

Preference will be given to applicants with experience in one or more of the following fields: digital communications, microwaves, medical electronics, electromagnetism, fundamental physical electronics.

Salary scales: Lecturer £3,883 to £7,754; Tutorial Fellow £3,883 to £6,555.

Application forms and particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH, to whom completed applications should be returned by March 12, 1979. Please quote: Ref. A4/79. 9565(A)

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF OCCUPATIONAL HEALTH AND HYGIENE

RESEARCH OFFICER

Applications are invited from Honours graduates, preferably with Ph.D., for a post as Research Officer in Applied Physiology which has been established in the Department of Occupational Health and Hygiene. The person appointed will be required to assist and collaborate in studies of human respiratory and exercise physiology including physiological anthropometry, control mechanisms, sports physiology and the effects of occupational dusts and vapours. Applications from holders of HNC in physiology, medical physics or applied biology with relevant experience will also be considered.

Salary according to age, qualifications and experience on either the Range 1B salary scale £3,384 to £4,882 per annum or the Range 1A salary scale £3,883 to £6,555 per annum. The post is available for up to three years. Membership of the appropriate University superannuation scheme will be required.

Applications (three copies) together with the names and addresses of three referees should be sent to The Senior Administrative Assistant, The Medical School, University of Newcastle upon Tyne NE1 7RU, not later than March 12, 1979. 9566(A)

The Physiological Institute, Lehrstuhl 2, University of Goettingen, is offering a contract for the position of **COMPUTER PROGRAMMER** for its PDP-11 computer network. A knowledge of digital electronics would be advantageous. Application with curriculum vitae to: H. Schultens/EDV, Humboldtallee 7, D-3400 Goettingen, FRG. 9575(A)

SENIOR HISTOPATHOLOGIST

[HE ST GEORGE HOSPITAL

with conjoint appointment as Clinical Professor or Clinical Associate Professor

School of Pathology

University of New South Wales

Australia

Suitably qualified medical pracitioners are invited to apply for ppointment as Senior Histopathologist t this 550 bed teaching hospital of the Jniversity of New South Wales. The ppointee may also be offered the title f Clinical Professor (conjoint appointnent) or Clinical Associate Professor conjoint appointment) and as such vould be responsible to the University f New South Wales for teaching in he School of Pathology, with access o the facilities of that school.

The Histopathology Division provides comprehensive service to the Hospital nd is staffed with a second full-time pecialist and two Registrars. A fullime Senior Lecturer in Pathology is lso on site.

Salary is at the Senior Staff Specialist rate, currently $A39,364 p.a. Other onditions in terms of the Medical Officers' Hospital Specialists' (State) Award include study leave of three nonths for each five years' service nd limited right of private practice n terms of the procedures laid down y the Health Commission of New South Wales.

Written applications, including the iames and addresses of three referees lose with the Chief Executive Officer, The St George Hospital, Kogarah, 2217, on April 12, 1979. Enquiries concerning hospital duties should be directed to the Medical Superintendent, The St George Hospial, phone (02) 588 1111, and on Uiversity matters to Professor A. Lykke (02) 662 2770.

9537(A)

UNIVERSITY OF HONG KONG

DEPARTMENT OF GEOGRAPHY AND GEOLOGY

Applications are invited for the post of

LECTURER/ ASSISTANT LECTURER IN ENGINEERING GEOLOGY

Candidates will be required to conluct courses in Geology for Engineerng students but will also be required o contribute in the Geology programme for Geography students.

Preference will be given to candidates with special qualification in Engineerng Geology, and with experience in he East and South East Asian region.

Annual salaries (superannuable) are: Lecturer HK$63,540 by 4,260 to 72,060 BAR 76,320 by 4,260 to 106,140; Assistant Lecturer HK$46,260 by 4,320 to 59,220. (£1=HK$9.40 approx.) Starting salary will depend on qualifications and experience.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or the Assistant Secretary (Recruitment), University of Hong Kong, Hong Kong.

Closing date for applications is **March 15, 1979.** 9570(A)

UNIVERSITY OF DURHAM

DEPARTMENT OF ZOOLOGY

Applications are invited for the post of

LECTURER IN ANIMAL BEHAVIOUR

tenable from October 1, 1979. Candidates should have field experience and be able to assist with ecological teaching and with the development of Joint Honours courses with Psychology and Anthropology.

Salary on the scale £3,883 to £7,754 per annum (under review) plus U.S.S. Applications (3 copies) naming three referees, should be sent by February 23, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained.

9463(A)

Radioimmunoassay Development

Our Radioimmunoassay kits are well established in the Haematology, Obstetrics/Gynaecology and Thyroid fields and make a substantial contribution to the company's 20% p.a. growth rate and current group turnover of more than £33m.

The group responsible for their development is expanding to meet the need for new products and we now have opportunities for experienced and skilled Radioimmunoassay Scientists to play a key role in our future development programme. Candidates must have appropriate experience in a commercial or specialist research environment and good academic training to degree level with a period in further research in Chemistry, Biochemistry or related subjects. Ability and success in developing Radioimmunoassay or related analytical methods should have been a strong feature in the career of the successful candidate. 1979 graduates (first or second degree) are unlikely to have sufficient experience.

The company is based in rural Buckinghamshire and this post offers considerable scope for scientists keen to join a successful and rapidly-expanding high technology organisation. Salaries will be in the range £5,600 – £7,600 depending on the qualities and experience of candidates. Conditions of employment are excellent and assistance with relocation will be given where needed.

In the first instance, please write with full details of career to: Dr. J. S. Allinson.

The Radiochemical Centre
Amersham Bucks
1974

9596(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF ANIMAL PHYSIOLOGY AND NUTRITION

Applications are invited for a temporary post of RESEARCH FELLOW in the above Department for work on a project, supported by the Agricultural Research Council, involving a study of phosphate homeostasis in sheep. Candidates should have a good honours degree in agricultural animal science, veterinary science or physiology and a Ph.D. degree. The appointment will be made for a fixed period of twenty months with effect from April 1, 1979.

Salary in the range £3,883 to £5,604 on the IA scale for Research and Analogous Staff (£3,883 to £6,555) (under review) according to age, qualifications and experience.

Applications forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 61/5/D. Closing date for applications, February 23, 1979.

9472(A)

As a result of expansion we have a vacancy for a

DESK EDITOR

for work on highly specialized scientific journals. Candidates must have had university level training in the Physical Sciences or Engineering. A concern for literate communication and excellent knowledge of the English language are essential, as is the ability to work precisely and efficiently under pressure. Previous publishing experience is necessary. Excellent prospects for the right candidate.

Applicants should contact the Editor:
Editorial Office (U.K.) of Elsevier Sequoia,
Mayfield House, 256 Banbury Road,
Oxford OX2 7DH.

ELSEVIER 9542(A)

SCHERING erwartet Sie

Im Department Entzündungspharmakologie unserer Pharma Forschung ist die Stelle eines(r)

Immunologen/ Immunologin

zu besetzen

Wir gehen davon aus, daß sich aus den Erkenntnissen der immunbiologischen Grundlagenforschung Impulse zur Therapie bisher unzulänglich behandelbarer Erkrankungen ergeben werden.

Wir wünschen uns einen Mitarbeiter, der aufgrund seiner mehrjährigen experimentellen Erfahrung in der Lage ist, sich an dieser Grundlagenforschung zu beteiligen. Sein Arbeitsgebiet wird die Regulation der Immunantwort auf zellularer Ebene sein; dort wird die Basis für eine spätere Etablierung von Modellen zur Wirkstoffindung gelegt.

Wenn Sie an dieser Aufgabenstellung interessiert sind, bewerben Sie sich bitte schriftlich mit den üblichen Unterlagen oder rufen Sie uns an (Tel.: 030/468 2853 oder 468 2922).

SCHERING AKTIENGESELLSCHAFT
Personalabteilung Berlin
Müllerstraße 170-178, 1000 Berlin 65

SCHERING AG

9528(A)

UNIVERSITY OF MANITOBA

DIVISION OF GENETICS, FACULTY OF MEDICINE AND HEALTH SCIENCES CENTRE

ASSOCIATE *or* ASSISTANT PROFESSORS

in areas of :

1. Clinical Genetics—M.D., F.R.C.P. or equivalent in Pediatrics. At least 3-4 years experience in Clinical Genetics/Genetic Counselling and Research to head Section of Clinical Genetics, Health Sciences Centre.
2. Basic Genetics—Ph.D. or M.D. with Research interests in the area of Somatic Cell Genetics, Population Genetics or Immunogenetics. Some experience in Graduate and Undergraduate teaching required.

Both appointments to commence on or after July 1, 1979. Send C.V. and references as soon as possible to Dr John L. Hamerton, Division of Genetics, Health Sciences Centre, 700 William Avenue, Winnipeg, Manitoba, R3E 0Z3. 9559(A)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF BIOLOGY

Applications are invited for the post of Lecturer in the Department of Biology. Candidates should have a special interest in Plant Ecology and should expect to contribute fully to the general teaching in the Department.

Salary scale £3,883 to £7,754 per annum plus U.S.S. Initial salary will depend on qualifications and experience.

Further particulars may be obtained from Mrs E. C. P. Sears, Staffing Section, The University, Highfield, Southampton SO9 5NH, to whom applications (seven copies), naming three referees should be sent, as soon as possible and not later than March 9, 1979. Please quote reference number 1609/R/Na. 9362(A)

UNIVERSITY OF LONDON ROYAL POSTGRADUATE MEDICAL SCHOOL

SAFETY OFFICER

Applications are invited for this newly established post, the holder of which will be Secretary of the Safety Committee and will be responsible to the School Secretary for the implementation of safety policy.

Applicants should have a sound knowledge of safety matters. Particular knowledge of safety considerations in laboratories would be an advantage, but is not essential.

Salary on grade II for academic-related Administrative Staff: £6,317 to £7,754 (under review) plus £450 a year London Allowance.

Superannuation under Universities Superannuation Scheme.

Further particulars should be obtained from the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS (tel: 01-743 2030, ext 352), to whom applications (6 copies) should be sent, naming three professional referees, to arrive not later than Wednesday, February 28, 1979. 9594(A)

GRADUATE or POSTDOCTORAL RESEARCH ASSISTANT required for 1¼ years in the Department of Microbiology for an M.R.C. research project on cytomegalovirus immunovirology. Laboratory experience in virology essential. Applications, with full curriculum vitae and the names of two referees, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. M.C.2. 9553(A)

POSTDOCTORAL RESEARCH POSITION immediately available for a young Ph.D. in Biochemistry interested in working on Interferon. The project includes specific aspects of the molecular biology of induction and mechanism of action of Interferon. Experience in nucleic acid research including hybridisation and cell-free translation would be helpful. Applicants should send their curriculum vitae, bibliography and the name and address of two referees to: Dr Jean CONTENT, Institut Pasteur du Brabant, 28 ru de Remorqueur, B 1040-BRUSSELS, BELGIUM. 9558(A)

Ph.D. PHYSIOLOGIST

with special interest in reproductive physiology wanted to direct gynecologic endocrine laboratory at Tufts New England Medical Center Hospital. Send curriculum vitae to: Martin Farber, M.D., Associate Professor—Department of Obstetrics and Gynecology, Director—Division of Reproductive Endocrinology, Tufts New England Medical Center Hospital, 37 Benner St, Boston, Mass. 02111.

An Equal Opportunity Employer M/F. 9500(A)

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR

Applications are invited for the post of LECTURER IN MATHEMATICS. Applicants should have research experience and publications in Pure Mathematics, preferably in an area of TOPOLOGY.

The appointment, to commence on October 1, 1979, will be to the Universities' Lecturer scale:—£3,883 to £7,754 per annum.

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained.

Closing date for applications: February 28, 1979. 9412(A)

SWINBURNE COLLEGE OF TECHNOLOGY

Hawthorn, Victoria, Australia

Applications are invited for appointment as

DEAN

FACULTY OF APPLIED SCIENCE

The appointment will be made either by contract, for at least three years, or on a permanent basis. If necessary, appropriate repatriation arrangements will be made for a contract appointment.

Swinburne is a tertiary institution currently affiliated with the Victoria Institute of Colleges, and with a large measure of autonomy. The Faculty of Applied Science offers courses at undergraduate and postgraduate levels in fields of Applied Chemistry, Biochemistry, Biophysics, Computer Science, Instrumental Science, Mathematics and Environmental Health. It has an academic staff of about 60 and approximately 550 cooperative (sandwich) full-time and part-time students.

Qualifications: Candidates should hold formal qualifications, preferably at postgraduate level, in a field of science appropriate to the activities of the Faculty. In addition, they should have had considerable experience in professional, industrial or research activities and in tertiary education at a senior level.

Salary: $A33,059 (current exchange rate $A1=£0.57 Stg. approximately).

Duties: The successful applicant will exercise responsibility for academic leadership and administrative coordination in the Faculty of Applied Science. The Dean is expected to liaise with outside industrial and professional groups.

Further information about the position, conditions of employment and application procedure may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Closing date: **April 6, 1979.** 9571(A)

HAMBURG UNIVERSITY

West Germany

TECHNICIAN

required to join a team involved i polynucleotide chemistry and bio chemistry. The post is particularl suitable for a person who would no like to do routine work but to tak part in an interesting and intensiv research programme. Experience wit nucleic acid chemistry (oligonucleotide synthesis) is desirable.

Salary: DM 25.000 per annum.

Personal details and details of qualifications, experiences and curriculun vitae should be addressed to Professo Dr H. Köster, Universität Hamburg 2 Hamburg 13, Martin-Luther-King Plaz 6 (Institut für Organische Chemi and Biochemie). 9573(A)

UNIVERSITY OF SURREY

DEPARTMENT OF BIOCHEMISTRY

RESEARCH OFFICER

Applications are invited for th position of Research Officer to wor on a project concerned with tumour of the central nervous system Applicants should be graduates o equivalent and have some experienc in biochemical analytical technique preferably including radioimmuno assay and fluorimetry.

The appointment is for one year a salary on the Range 1B scale (£3,38 to £4,882 per annum).

Applications in the form of curriculum vitae, including the name and addresses of two referees, shoul be sent to the Staff Officer, Universit of Surrey, Guildford, Surrey, GU 5XH, by March 2, 1979. Furthe details may be obtained from the Sta Officer on Guildford 71281, ext. 45 9567(A)

MERCK SHARP & DOHME CHIBRET

a subsidiary of one of the leading pharmaceutical research organization
is seeking a

director of pharmacology

The person appointed will report to the Director of Research and will lead a Research team of 15 scientists and technicians presently working in the following fields :

- OCULAR PHARMACOLOGY

- BIOCHEMICAL PHARMACOLOGY

- PHARMACOKINETICS AND DRUG METABOLISM

The applicant should have a Ph.D. level or equivalent qualification and proven expertise pharmacological and biochemical Research.

A practicing knowledge of both written and spoken French and English is required.

The new, modern Research facility is located in pleasant surroundings at RIOM, FRANCE in the PUY-DE-DOME.

MSD
MERCK
SHARP&
DOHME
CHIBRET

For confidential consideration please send a detailed resume with curriculum vitae to
THE DIRECTION DU PERSONNEL
200, Boulevard Etienne-Clémentel
63018 CLERMONT-FERRAND CEDEX (FRANCE)

9560(A)

FREE UNIVERSITY AMSTERDAM

Applicants are invited
for the position of

PROFESSOR OF GEOMETRY

His main tasks will be to do research and to teach (and to stimulate and conduct both research and teaching) in an area of geometry which links up with the research in at least one of the fields of analysis, set-theoretical topology or algebra which is going on in the department A first preference will be given to those mathematicians for whom differential geometry is part of their research interest, but also other geometers are invited to apply

The mathematical department of the Free University has a student enrolment of approximately 300 and has 13 professors in pure and applied mathematics and 25 further staff members.

The Free University of Amsterdam, the Netherlands, is a fully state-subsidised, private university, and was founded as a non-denominational Christian institute. Its charter speaks of the Christian idea of service as the University's guiding principle Applicants are invited to state their position with respect to this principle

If desired more information can be obtained by writing to Professor M A Maurice, Wiskundig Seminarium der Vrije Universiteit, De Boelelaan 1081, Amsterdam, The Netherlands

Applicants are requested to submit, within three weeks after the appearance of this issue, curriculum vitae and list of publications, mentioning number 310-2458: dienst Personeelszaken, Vrije Universiteit, P O Box 7161, 1007 MC Amsterdam, The Netherlands. 9502(A)

COMMONWEALTH AGRICULTURAL BUREAUX

COMMONWEALTH INSTITUTE OF BIOLOGICAL CONTROL

Vacancy for a SCIENTIFIC INFORMATION OFFICER

London SW7

Duties: To assist in the preparation of a new journal containing abstracts and news items of interest to pest control scientists employing biological control This will include selection of relevant abstracts prepared by other CAB units, writing new abstracts and news items, and providing information in response to enquiries.

Location: The officer will be accommodated at the Commonwealth Institute of Entomology, 56 Queen's Gate, London SW7, and work alongside the staff of that Institute.

Qualifications: An honours degree in biology and preferably experience in pest control. A knowledge of entomology would be an advantage, as would a working knowledge of one or more foreign languages. The ability to write good English is essential

Salary: In the scale £2,839 to £5,448 per annum plus Inner London Weighting of £465 per annum, plus a compensatory allowance (taxable but not superannuable) of 3.3% to offset personal contribution to Superannuation Scheme. Starting salary according to qualifications, experience and age

Application forms and full particulars from the Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN, to whom completed applications (in duplicate) should be sent by March 15, 1979. 9582(A)

TEACHING APPOINTMENTS

UNIVERSITY OF SINGAPORE
and
NANYANG UNIVERSITY

Applications are invited for teaching appointments, ranging from Lectureships to Professorships, in the following departments of the University of Singapore and Nanyang University.

University of Singapore

(i) *Department of Botany*—Candidates should have a Ph D. degree in Plant and/or Animal Biochemistry, with a good knowledge of ultra structure reseach, cytology, cytogenetics and biological functions. Preference will be given to candidates with some teaching and research experience in these fields.

(ii) *Department of Chemistry*—Candidates should have a Ph D degree in Chemistry, preferably with specialisation and teaching/industrial experience in applied chemistry.

(iii) *Department of Zoology*—Candidates should have a Ph D degree in molecular and cell biology, developmental biology, or marine biology

Nanyang University

Department of Chemistry—Candidates should have a Ph D degree in any field of inorganic chemistry, preferably in organometallic chemistry Knowledge of industrial or environmental applications will be an advantage.

Gross monthly emoluments in the range from S$1,445 to S$5,775 approx, point of entry depending on the candidate's qualifications and experience and level of appointment offered. In addition, the University pays a 13th month annual allowance of one month's salary in December of each year Leave, subsidised housing, medical and provident fund benefits are available FOR ALL APPOINTMENTS, SUCCESSFUL APPLICANTS MUST BE PREPARED TO TEACH AT BOTH UNIVERSITIES Candidates should write to the Registrar, University of Singapore, Kent Ridge, Singapore 5, or the Registrar, Nanyang University, Upper Jurong Road, Singapore 22 (as applicable) giving curriculum vitae (bio-data) with full personal particulars and also the name and addresses of three referees (Candidates should duplicate applications if they wish to apply to both Universities.) 9536(A)

Graduate Biochemist

The Pharmaceuticals Division of Imperial Chemical Industries is seeking a graduate biochemist to join a research team that is investigating abnormalities of hormonal regulation in disease processes The post requires a biochemist with a high level of initiative and commitment to the application of biochemistry to drug research Applicants should have a minimum of two years post graduate laboratory experience

Our modern research laboratories are situated in pleasant surroundings, but within easy reach of main road and rail routes

Conditions of service, salary and assistance to married staff in moving house are designed to attract and retain staff of high calibre.

Please write giving details of qualifications and experience to:

Mrs. D. Bass, Assistant
Personnel Officer, Imperial
Chemical Industries Limited,
Pharmaceuticals Division,
Mereside Research
Laboratories, Alderley Park,
Nr. Macclesfield, Cheshire

9554(A)

M.R.C. CLINICAL RESEARCH CENTRE

(Northwick Park Hospital)
Watford Road, Harrow
Middlesex HA1 3UJ

A SENIOR POSTDOCTORAL PHYSIOLOGIST

This is a new appointment to work with Dr Michael Halsey on high pressure studies in non-human primates The successful applicant will take a major part in the development of a national pressure chamber facility which will be used for studying the high pressure neurological syndrome and its amelioration by anaesthetics

Potential applicants are invited to visit informally the Clinical Research Centre and to discuss their research ideas with Dr Halsey before applying

This is a limited term appointment and the salary will be in the range £4,832 to £6,530 (including London Allowance). Dr Halsey can be contacted for further details (tel 01-864 5311, ext 2452) and application forms are obtainable from Mrs J Tucker-Bull, quoting ref No 101/1/4281 9544(A)

UNIVERSITY OF LONDON
KING'S COLLEGE
LECTURESHIPS IN GEOGRAPHY

Applications are invited for appointment to TWO additional Lectureships to be provided in the Department of Geography at King's College with effect from the 1 October, 1979 Any areas of specialisation will be considered, although duties will involve assistance with quantitative practical classes The lecturers appointed will be under the age of 30 in order to preserve a balanced age structure among departmental staff.

Salary will be in the range £3,883 to £7,754 per annum (under review), plus £450 per annum London Allowance. Universities Superannuation Scheme contributions will be payable

Application forms and general information on the Department may be obtained by writing to The Assistant Registrar, University of London King's College, Strand, London WC2R 2LS Applications, in triplicate, including a detailed Curriculum Vitae, should reach the Assistant Registrar by March 1, 1979, quoting reference 191656/N. 9513(A)

UNIVERSITY OF LONDON
INSTITUTE OF PSYCHIATRY
A GRADUATE RESEARCH ASSISTANT

is required to join the London team of the Cross National (U.S./U.K.) Study This study is investigating the outcome for selected elderly people receiving residential or day care in London and New York It is the latest in a series of studies conducted by multidisciplinary teams in the two cities.

The post will involve (a) participation in fieldwork, particularly in the collection of data using behavioural observation methods and (b) assisting with the preparation for analysis of interview and observation data It is likely to be of interest to graduates in the behavioural or social sciences who wish to gain experience in social and epidemiological research methods

Those wishing to work part time will be considered for this post.

Salary £3,384 to £4,882 plus £450 London Weighting

Application forms and enquiries. Mrs B Robinson, 7 Windsor Walk, London SE5 Telephone 01-703 0417. 9515(A)

BEDFORD COLLEGE
(University of London)
HISTOLOGIST TECHNICIAN (GRADE 5)

required in the
DEPARTMENT OF ZOOLOGY

to assist in the research of Academic Staff both with routine histology and the preparation of materials for electron microscopy. The person appointed will be expected to assist with undergraduate practical classes and postgraduates requiring instruction in histological techniques. Previous experience is essential

Salary range (under review) £3,186 to £3,720 per annum plus £465 per annum London Weighting 25 days annual leave plus statutory holidays Season ticket loan scheme

Application forms available from the Senior Assistant Secretary (Personnel), Bedford College, Regent's Park, London NW1 4NS (Tel. 01-486 4400, Ext 312) 9514(A)

UNIVERSITY OF SOUTHAMPTON
MEDICAL ONCOLOGY UNIT

Applications are invited for the post of Research Assistant in the Medical Oncology Unit at Southampton General Hospital to work on immunological aspects of cancer and other diseases Preference will be given to candidates who have either some experience of immunological and radio-isotope techniques or an honours degree in biochemistry or a related subject The successful applicant will be expected to start as soon as possible

The post is available for 2½ years in the first instance

Salary on scale 1B, £3,384 to £4,882 per annum

Applications (three copies) should be sent to Mrs P Vaughan-Smith, The University, Southampton SO9 5NH, as soon as possible quoting reference 1054/R/Na 9504(A)

GRAVITATIONAL–WAV
DETECTION AND
EXPERIMENTAL
GRAVITY—CALTECH

The California Institute of T nology is creating a new resea group in gravitational wave detec and experimental gravity Various vestigations related to gravitatio radiation are being planned, u laser interferometry, cryogenic e tronics, or other techniques, and related experiments over a 7,600 k metre baseline between California Scotland may be performed group will be in a good position close collaboration with Calte gravitation theory group, and also pursuing gravity experiments in sp in association with Caltech's Propulsion Laboratory.

Inquiries are invited from outsta ing experimental physicists, engine or technicians who might wish to this new group It is anticipated the following types of appointm may be made in 1979 and 1980. search staff at levels equivalent assistant, associate, or full profe but without teaching duties, assis professorships, postdoctoral fell ships, and technicians Inquiries f women and minorities are particul encouraged

Inquiries — including curricul vitae, lists of publications, and let of recommendations sent independe —should be sent to Professor Ro W P Drever, 130-33 Caltech, P dena, California 91125, or Departm of Natural Philosophy, Glasgow U versity, Glasgow G12 8QQ, Scotl 9411(A

UNIVERSITY OF OXFOR
DYSON PERRINS (ORGANIC CHEMISTRY) LABORATORY
DEPARTMENTAL ASSISTANT

Applications are invited f organic chemists of postdoctoral st for the post of Department Assist from September 1, 1979

The salary will be on the s £3,883 to £4,882 per annum accord to age and experience and membership of U S S

The appointment will be for period not exceeding two years the successful applicant will expected to collaborate with one the research groups and super undergraduate experiments

Further particulars may be obta from the Administrator, Dyson Per Laboratory, South Parks Road, Oxf OX1 3QY, to whom applications the names of two referees should sent by February 28 9398(A

UNIVERSITY OF BIRMINGHAM
Dental School
DEPARTMENT OF ORAL PATHOLOGY
RESEARCH FELLOW

Postdoctoral Research Fellow quired to continue a project into development of *in vitro* methods testing the toxicity of dental mater Previous experience of cell cult and quantitative cytochemical te niques would be an advantage, bu not essential The project is for th years and would be carried out parallel with other *in vitro* proj currently in progress

Salary on the scale £3,883 to £6, plus superannuation

Further particulars available f the Assistant Registrar, The De School, St Chad's Queensway, mingham B4 6NN, to whom ap cations, including a full curricul vitae and the names of three refe should be sent by February 28, 1 9370(A

free university
amsterdam

The **Faculty of Mathematics and Natural Sciences**, experiments with an advanced teaching programme on "contemporary problems in the present relation between science and society, of which the results may be relevant to problems of science policy."
The teaching focusses on energy problems Physical and Chemical expertise is given by the respective departments. There is a vacancy for a specialist on social and economic aspects of the energy problems, within the **Unit for the History and Social Aspects of Science:**

lecturer

(up to 31 December 1980)

Task:

- teaching social and economic aspects of the energy problem
- maintaining contacts with the science departments

Requirements:

- expertness in socio-economic aspects of the energy problems
- research experience and publications
- teaching ability in this field

The Vrije Universiteit is a fully state-supported university with a Christian foundation: candidates are expected to indicate their willingness to respect, and work in the spirit of the Christian character of the university.
Salary between Dfl. 48.000 and 85.000. Applicant may start immediately. Information on the content of the teaching programme will be given by prof dr. E Boeker, tel 020 - 548 47 36

All further information will be given by the chairman of the Unit, prof.dr. M.J.S. Rudwick, tel. 020 - 548 29 86
Written applications with curriculum vitae, list of publications and 2 references should be sent to dienst Personeelszaken van de Vrije Universiteit, Postbus 7161, 1007 MC Amsterdam, vacancy nr. 380-0019.

9503(A)

MEDICAL RESEARCH COUNCIL
Laboratory Animals Centre

NUTRITIONIST

Scientists with postdoctoral research cperience in the nutrition of labora- ry animals, farm livestock or poultry e invited to apply for the post of ead of the Centre's Nutrition Depart- ent The successful applicant will be e focus of all aspects of the entre's work in this field and will ndertake research into the nutritional quirements of laboratory animals ading to general improvements in utrition with particular emphasis on reeding performance and the lowering f mortality rates He or she will also rovide a comprehensive advisory rvice to diet manufacturers and users f laboratory animals

Excellent conditions of service, tracting a salary commensurate with e, qualifications and relevant experi- ice.

Applications, enclosing a full urriculum vitae, should reach the irector, Laboratory Animals Centre, ledical Research Council Laboratories, 'oodmansterne Road, Carshalton, urrey SM5 4EF by March 1, 1979

9501(A)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a temporary post of RESEARCH FELLOW in the above Department from biochemists or chemists with an interest in metalloproteins to work on a project, supported by the Science Research Council, on the equilibria and kinetics of oxygen binding by respiratory proteins and their subunits in collaboration with Dr E J Wood Candidates should possess a Ph D degree. The appointment will be made for a fixed period of up to three years

Starting salary in the range £3,883 to £4,382 on the 1A scale for Research and Analogous Staff (£3,883 to £6,555) under review) according to age, qualifications and experience.

Informal enquiries may be addressed in the first instance to Dr E J. Wood (telephone. (0532) 31751, Extn 66)

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 83/18/D Closing date for applications: February 26, 1979.

9476(A)

AGRICULTURAL RESEARCH COUNCIL

POULTRY RESEARCH CENTRE
King's Buildings, West Mains Road
Edinburgh EH9 3JS, Scotland

HIGHER SCIENTIFIC OFFICER

Applications are invited for the post within the H S O grade, Band II in the Reproductive Physiology Department at the above Centre.

Initially the successful candidate will be required to participate in the development and use of in vivo and in vitro systems to study lipogenesis, lipoprotein metabolism and related metabolic processes in relation to the production and control of egg-yolk constituents by the liver of the domestic fowl.

Previous experience in the use of enzymatic assay techniques, determination of intermediary metabites and methods of protein analysis is desirable

Qualifications Degree, H N.D./H N C or equivalent in Biochemistry/Chemistry with at least 5 years relevant post qualifying experience. Knowledge of related disciplines, e.g. physiology, endocrinology would be an advantage

Salary. In scale £4,101 to £5,448 22 days annual leave plus 10½ days public/privilege holidays A non-contributory pension scheme is in operation

Application forms may be obtained from the Assistant Secretary at the above address These should be returned to her not later than February 28, 1979 Please quote Reference No. PRC 795. 9557(A)

CENTRAL ELECTRICITY RESEARCH LABORATORIES

Kelvin Avenue, Leatherhead, Surrey, KT22 7SE

RESEARCH OFFICER

ENVIRONMENTAL PHYSICS SECTION

The Environmental Physics Section, whose role at CERL is to study the physical, meteorological, and mathematical aspects of the dispersion and fate of atmospheric emissions from power stations, requires a graduate/postgraduate physicist to undertake research on certain aspects of the global CO_2 problem These relate to the takeup of CO_2 at natural surfaces such as vegetation, forests, and the sea, as part of a programme to study the ground level deposition of airborne species like SO_2, NO_x, CO_2, and particulates. Accurate methods for measuring the flux of these species to ground need to be developed to study the CO_2 balance, the effects of air pollution on crops, and the transport of pollution over continental distances

The successful candidate would be expected to develop the necessary techniques, apply them in field experiments, and establish an informed position on the overall CO_2 problem. He/she would be working with other scientists concerned with the dispersion of emissions from power stations whose facilities include the latest mobile remote sensors, ground samplers, meteorological instrumentation, and data processing

Candidates should have a good honours degree in physics, preferably with post-graduate experience in a relevant field such as environmental science or meteorology

The Laboratories are situated in a pleasant part of Surrey and offer attractive conditions of service, and facilities for the total of 800 Research and Support staff engaged in a broad spectrum of research into the materials, technologies and plant performance problems of the Central Electricity Generating Board

This appointment will be made within incremental salary ranges rising to £6035 or £7050 plus a Self-Financing Productivity Scheme payment ranging between £8 and £17 per month

Application forms are obtainable from the Head of Personnel Development & Services, Central Electricity Research Laboratories, at the above address or telephone Leatherhead 74488 Ext 363, quoting reference number RL/5. Closing date is **Friday 23 February 1979.** 9587(A)

CHAIRPERSON

Applications are sought for the position of chairperson of the Department of Veterinary Physiology and Pharmacology, Iowa State University Available July 1, 1979 Qualifications include the DVM and/or Ph D degrees, academic leadership potential, and demonstrated excellence in teaching and research

Send applications, including curriculum vitae and bibliography by April 1, 1979, to Dr F Ahrens, Department of Veterinary Physiology and Pharmacology, Iowa State University, Ames, Iowa 50011

An Affirmative Action/Equal Opportunity Employer 9543(A)

YARMOUK UNIVERSITY

Irbed—Jordan

invites applications for teaching positions for the academic year 1979-1980 in the following areas

Biological Sciences Embryology-Comparative Anatomy, Animal Physiology, Microbiology, Plant Pathology, Taxonomy-Ecology, Plant Physiology

Appointments will be at the level of Assistant Professor, Associate Professor or Professor Applicants must hold a Ph.D. Placement will be according to experience in teaching and research Salaries range· $16,000 to $27,500 (9 months). Fringe benefits include travel expenses at beginning and termination of employment and for home leave every three years for appointee and members of family, health and life insurance, and furnished accommodation at reasonable cost. 9523(A)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL BIOENGINEERING

Applications are invited for a newly-established post of LECTURER IN BIOENGINEERING in the Department of Surgery The Lecturer will work in close collaboration with the N H S Department of Medical Physics, Queen's Medical Centre, Nottingham

The successful candidate will be expected to have special experience in bioengineering and computer analysis, and will help to extend collaboration between departments in the Medical School and the Department of Medical Physics

Salary will be within the non-clinical scale for lecturers with membership of U S S/F S S U. as appropriate. The successful applicant will be expected to take up the appointment as soon as possible

Those interested in this post are invited to write for details and a form of application to the Deputy Registrar, Medical School, Queen's Medical Centre, Nottingham NG7 2UH to whom completed applications should be returned by March 1, 1979

9469(A)

POSTDOCTORAL POSITION available for a 2-3 year study of the role of prostaglandins and cyclic nucleotides in stimulus-secretion coupling Curriculum vitae and two letters of recommendation should be sent to: Dr R. P Rubin, Department of Pharmacology, Medical College of Virginia, Richmond, Virginia 23298, U S A

9524(A)

HYDROGAPHER

The University of Delaware is seeking a professional hydrogapher This person will have a principal role in procurement, development and testing of a new oceanographic CTD system which will include shipboard digital data storage and computer processing After system development the hydrographer will be responsible for its use in data collection at sea about 50 days per year and for data processing, sensor calibration and routine system maintenance ashore The position requires a M S in physical or chemical oceanography or equivalent Shipboard experience with CTD systems, expert knowledge of computer processing, and basic knowledge of digital electronics and computer interfacing are desired Salary commensurate with experience and ability.

Send résumé to Mr Wadsworth Owen, Uiversity of Delaware, College of Marine Studies, P O Box 286, Lewes, DE 19958

The University of Delaware is an Affirmative Action/Equal Opportunity Employer 9525(A)

UNIVERSITY OF MALAW

Bunda College of Agriculture

Applications are invited for th following posts in the DEPAR MENT OF LIVESTOCK PRODU TION.

LECTURER IN LIVESTOCK NUTRITION

Applicants should have a good d gree in Agriculture, or Anim husbandry or a related descipline, wi post-graduate qualification in Liv stock Nutrition. Practical experien in livestock management would be advantage. The appointee's major re ponsibility will be to teach appli livestock nutrition to Degree a Diploma students who will have take courses in Agricultural Chemistry a Biochemistry. There are opportunit for research

LECTURER IN ANIMAL HEALTH

Applicants should have a degree other recognised University level qu lifications in Veterinary Science (su as M R.C.V.S.), or alternatively basic degree in Biology or Agricu tural practical experience in the han ling and treatment of livestock. Dut will include teaching courses in Anim Health, and Management, with refe ence to diseases and parasites Africa, practical demonstration, an research directed toward the improv ment of animal health and productio under local conditions

Salary scale: Lecturer K3,500 K6,600 p a (£1 sterling to K1 62). T British Government may suppleme salary in range £3,450 to 3,732 p. (sterling) for married appointees an £2,340 to £2,544 p.a (sterling) f single appointees (reviewed annuall and normally free of tax) and provi children's holiday visit passages an education allowances If no Britis Government supplement is availabl the University may pay an addition between K1,320 to K1,656 p a (taxab in Malawi). Family passages, supera nuation scheme, gratuity 15-25 biennial overseas leave, housing an various allowances Detailed applic tions (2 copies) with curriculum vit and naming 3 referees to be sent Registrar, University of Malawi, P Box 278, Zomba, Malawi by Marc 22, 1979 Applicants resident in t UK should also send one copy to I ter-University Council, 90/91 Totte ham Court Road, London W1P 0D Furher details may be obtained fro either address 9479(A)

ROTHAMSTED EXPERIMENTAL STATION

Harpenden, Herts. AL5 2JQ

PHYSICIST APPLIED PHYSICIST or ELECTRONICS ENGINEER

to support data acquisition phases Physics Department field experimen into the effects of tillage and droug on crop growth.

Qualifications. Degree, H N C equivalent in physics, applied physi or electronic engineering, wi adequate mathematical training an experience of electronic circuitry and or computer programming Acceptab post-qualifying experience is require for appointment to the higher grad Driving licence particularly useful.

Appointment in grade of Scientif Officer (£2,839 to £4,415 per annun or Higher Scientific Officer (£4,101 £5,448 per annum) Starting sala according to qualifications and exper ence Non-contributory superannuatio Apply in writing to the Secretar giving names and addresses of tw referees and quoting Ref. 386 March 1, 1979 Further details o request. 9492(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF IOCHEMISTRY & CHEMISTRY

TECHNICIAN is required, with erience of organic synthesis and ctroscopic techniques, to work with team studying biomembrane struc- and function.

alary on Whitley Council Scale.

Application forms, which are avail- e from the School Secretary, R.F. S.M., 8 Hunter Street, London, 1N 1BP (or telephone 01-837 5385 t. 10) should be returned by Feb- ry 22, 1979. 9464(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

pplications are invited from Ph.D. duates in Biochemistry with experi- e in protein or membrane bio- mistry for the post of Postdoctoral search Assistant to work on a ject financed by the S.R.C. entitled lation and characterisation of nine nucleotide transporters of yeast tant mitochondria'. The work will olve protein purification, protein icture determination on examination transport function in reconstituted teoliposomes. The appointment will tenable for up to two years from e 1, 1979 or as soon as can be anged.

nitial salary either £3,883 or £4,133 annum.

Applications, together with the nes of two referees, should be eived not later than February 26, '9 by The Registrar, The University,). Box 147, Liverpool L69 3BX, m whom further particulars may be ained. Quote Ref: 471/N. 9495(A)

NIVERSITY OF WARWICK GRADUATE RESEARCH ASSOCIATE IN PHYSICS

Professor P. N. Butcher is seeking a l qualified graduate to work with for three years on the theory of oping conductivity in amorphous iconductors. The post is supported the Science Research Council and tenable for three years starting in tober, 1979. Initial salary £3,384 annum on the Range 1B scale: 384, £3,634, £3,883 x £249 to 631, £4,882 per annum (under iew). Applications are invited from dents taking their final under- duate examinations in June 1979. e successful applicant will be ected to register for a Ph.D. Appli- ion forms from the Academic gistrar, University of Warwick, ventry CV4 7AL, quoting Ref. No. A/79; to be reurned as soon as sible. 9496(A)

NIVERSITY OF OXFORD UNIVERSITY LECTURERSHIP IN ZOOLOGY

pplications are invited for the ve post from candidates in the d of comparative physiology. end according to age on the scale 383 to £8,257 per annum. The cessful candidate may be offered a rial fellowship at St Hugh's Col- (open to men and women). ther details of both posts may be ained from the Acting Head of artment, Department of Zoology, th Parks Road, Oxford OX1 3PS, whom completed applications (nine d copies, one from overseas candi- s) including the names of three rees should be sent by March 9, 9. 9497(A)

TECHNICAL MARKETING EXECUTIVE. Whatman Biochemicals Ltd. require a young dynamic person to assist the Marketing Manager in various aspects of Enzyme Marketing. The successful applicant will eventually be responsible for selling diagnostic enzymes in Western Europe and will also be involved in investigating new markets for the company's enzyme products. Applicants should have a degree in a biological science, preferably biochemistry and a strong desire to follow a marketing career. Some marketing sales experience and a knowledge of German and/or Italian would be helpful. The successful applicant will probably be between 25 and 30 years. Applications should be made to Personnel, Maidstone, Kent. Tel: 0622 61681.

9494(A)

UNIVERSITY OF MANCHESTER

DEPARTMENTS OF BOTANY AND ZOOLOGY

SENIOR DEMONSTRATOR IN BIOLOGY

Applications invited from persons with a Ph.D. or good houours degree in one of the biological sciences and an interest in biological education. The main duties will be to co-operate in the organisation and development of 1st and 2nd year laboratory classes in Cell Biology, Cell Physiology, Genetics, Ecology and Data Analysis. Facilities for part-time research will be available. The appointment is for three years, commencing October 1979, and may be renewed for a second three year period. Initial salary within the range £3,883 to £4,631 p.a. Superannuation. Particulars and application forms (returnable by March 20th) from the Registrar, The University, Manchester, M13 9PL. Quote ref: 18/79/N. 9466(A)

University of London

READERSHIP IN PHYSIOLOGY AT QUEEN ELIZABETH COLLEGE

The Senate invites applications for the above Readership to be held at the Department of Physiology (Head of Department: Professor D. L. Yudilevitch, M.D.). The Reader will be an experienced physiologist of proven research ability. The Department has been expanding its research activities in the past few years and is now well-equipped for studies in its current interests. Queen Elizabeth College is located in Kensington in a pleasant neighbourhood near Holland Park.

The successful candidate will be expected to develop his/her own research, supervise postgraduate research students and to contribute to undergraduate teaching.

Applications (10 copies) must be received not later than March 30, 1979, by the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, from whom further particulars (including the salary scale) should first be obtained.

9451(A)

CELL BIOLOGIST, PATHOLOGIST, Ph.D.,

with 3 years of postdoctoral experience for cancer cells, organ culture and SEM, TEM techniques seeks position in hospital or industry for applied research, preferably in France or Switzerland. Reply to:

Box No. 9585(A)
Nature, 3 Dyers Buildings
London EC1N 2NR

9585(A)

RESEARCH CHEMIST

PHARMACEUTICAL INDUSTRY

Pfizer Central Research is part of a leading international group engaged in the research, development, manufacture and marketing of a wide range of human medicinal and animal health products.

We have a vacancy for a chemist to work in our Agricultural Chemistry Department. Preferred male or female applicants will have:–

- Qualifications at HNC, GRIC, or B.Sc. level.
- Several years experience in synthetic organic chemistry, preferably in the pharmaceutical industry.
- Proven ability as an experimentalist.
- The ability to work with minimal supervision.

In return we offer:–

- A competitive starting salary within an incremental pay system.
- Varied synthetic work supported by first class analytical and library facilities.
- Modern laboratories within easy reach of pleasant seaside resorts, the Kentish countryside and Continental Europe.
- Flexible working hours.
- Participation in Bonus, Salary Continuance, Pension and Death Benefit schemes.
- A flourishing sports and social club.

Pfizer

If you are interested in this opportunity write or telephone for an application form to:– **Graham Marshall, Personnel Officer, Pfizer Central Research, Sandwich, Kent. Tel: (03046) 3511**

9561(A)

LOTHIAN HEALTH BOARD

BASIC GRADE CYTOGENETICIST

Applications are invited from science graduates for the above post based at the Department of Pathology, Royal Hospital for Sick Children, Edinburgh. Experience in human cytogenetics desirable.

All types of cytogenetic investigation are undertaken including the prenatal diagnosis of chromosome disorders, blood culture analysis, bone marrow investigation and the karyotyping of postmortem and spontaneous abortion material. A broad training in cytogenetics would be afforded.

Arrangements to visit the department may be made by telephoning 031-667 1991, ext. 270.

Salary scale: £2,991 per annum to £4,899 per annum with placement according to qualifications and experience.

Applications, which should be typewritten, giving particulars of age, qualifications and previous experience, together with the names and addresses of two referees should be lodged with the Secretary, 11 Drumsheugh Gardens, Edinburgh EH3 7QQ by February 22, 1979.

9498(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY

Applications are invited for a post of

POSTDOCTORAL RESEARCH FELLOW

from inorganic or physical chemists. Candidates should already possess, or expect to receive within the next few months a Ph.D. degree. Experience in inorganic synthesis or pulse nmr would be valuable. The appointee will take part in a project, supported by S.R.C., and will prepare and study NEW SOLID STATE INORGANIC PROTON CONDUCTORS applicable to fuel or electrolysis cells, hydride batteries or electrochromic displays. The appointment will be for a fixed period of two years to commence as soon as possible or from October 1, 1979.

Starting salary in the range £3,883 to £4,382 on the 1A scale for Research and Analogous Staff (£3,883 to £6,555) (under review) according to age, qualifications and experience.

Informal enquiries may be addressed in the first instance to Dr A. T. Howe, Department of Inorganic and Structural Chemistry (Tel: 0532-31751, ext. 6061).

Applications (quoting reference number 44/5) should be made as soon as possible to the Registrar, The University, Leeds LS2 9JT (from whom further particulars may be obtained). 9581(A)

ZOOLOGY CHAIRMAN GUELPH

The College of Biological Science is searching for a Chairman. The department wishes to recruit a scholar with expertise in one of the areas of specialization of the department.

The department has a large undergraduate teaching component both as a service function as well as in its own disciplinary area. At the graduate level the department specializes in:— aquatic science, marine biology, parasitology, environmental physiology, fish and wildlife biology, management and behaviour.

Salary and rank are commensurate with experience.

Recommendations and applications should be sent to. Secretary, Selection Committee, c/o Professor K. Ronald, Dean, College of Biological Science, University of Guelph, Guelph, Ontario, N1G 2WI.

Position subject to final budgetary approval.

9526(A)

(Re-advertisement)

RESEARCH SCIENTIST

THE PHARMACOLOGY OF FEVER

The National Institute for Biological Standards and Control is a recently established multi-disciplinary Institute concerned with the standardisation and control of biological substances important in medicine

The Institute's Division of Hormones requires a research scientist for a three year appointment for work involving the mechanism of fever production, the action of bacterial pyrogens and the development of methods for the measurement of their effects The work will provide good opportunities for collaboration both in the UK and internationally.

Candidates should preferably possess a PhD although those with a good honours degree in pharmacology or a related subject will be considered. It is hoped that the person appointed will be able to take up the post as soon as possible

Salary will be in accordance with the scales for university non-clinical lecturing staff and will initially be in the range **£4,300 to £6,500 p.a**

Please write or telephone, for an application form quoting Ref. No. 0138, to the Personnel Officer, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB Tel: 01-435 2232, ext. 211. Closing date: 23.2.79.

9562(A)

NIBSC

National Institute for Biological Standards and Control

UNIVERSITY OF EXETER
GEOLOGY DEPARTMENT
INSTITUTE OF GEOLOGICAL RESEARCH CONTRACT

A vacancy exists for

3 RESEARCH ASSISTANTS

with interests in Geological mapping, to work on the revision of Geological Survey Sheets 337 and 338.

The posts are tenable for one year in the first instance, from August 1, 1979, with the possibility of renewal thereafter

Successful applicants will be encouraged to register for a Higher Degree.

Commencing salary: £3,384 per annum (under review)

Further details from Dr E B. Selwood, Department of Geology, North Park Road, Exeter 9468(A)

UNIVERSITY OF MANCHESTER
DEPARTMENT OF PHYSICS
LASER RESEARCH

Applications are invited for a post of Research Associate in the Department of Physics for work on one of the following topics: laser coherent interactions with matter, new gas lasers or laser techniques. Previous research or development experience in related fields is desirable. The post is for two years in the first instance Initial salary within the range £4,382 to £5,604 per annum Superannuation. Application with curriculum vitae and the names of two referees should be sent to Dr T A King, Department of Physics, Schuster Laboratory, The University, Manchester M13 9PL

9467(A)

UNIVERSITY OF OXFORD
NUFFIELD DEPARTMENT OF PATHOLOGY AND BACTERIOLOGY
RESEARCH ASSISTANT

Graduate with good honours degree required to participate in a research project in the local and distant spread of cancer. The work involves tissue culture, enzyme isolation and purification, also studies with radioactive isotopes Experience in any of the above techniques will be an advantage

This work is supported by a grant from the Cancer Research Campaign. Salary: (Starting point) £3,634 with two anual increments to £4,133

Applications together with the names of two referees to be sent to Dr D Tarin, Clinical Reader in Pathology, Gibson Laboratories, Radcliffe Infirmary, Oxford, from whom further details can be obtained 9527(A)

THE UNIVERSITY OF HULL
DEPARTMENT OF PHYSICS

A vacancy exists for a Grade 3 technician for duties in a research group investigating luminescent compounds, involving the use of lasers and associated electronic and optical equipment Applicants should possess at least two science 'A' levels or O N C. and at least three years laboratory experience Salary on the scale £2,688 to £3,060 per annum (under review).

Applications, giving details of age, qualifications, experience and the names of two referees should reach the Technical Staff Officer, University of Hull, HU6 7RX, quoting Ref No TP/1, by February 19, 1979. Further particulars are available 9521(A)

WESTMINSTER MEDICAL SCHOOL
LEUKAEMIA RESEARCH
TECHNICIAN

Applications are invited from s
able persons for the post of Resea
Technician in the Department
Haematology at Westminster Hospi
The research concerns human b
marrow transplantation and treatm
of leukaemia The post gives the
portunity for obtaining the F I M I
degree by thesis The salary and c
ditions of employment are compar
to those agreed by the Whitby Coun
Applications should be sent as s
as possible to Dr A J Barrett,
partment of Haematology, Westmin
Hospital, Dean Ryle Street, S W 1
tending applicants may visit the lab
tory by appointment with Dr Barr
01-828 9811 Ext 2121 9550(A

MACDONALD CAMPUS C
McGILL UNIVERSITY
DEPARTMENT OF PLANT SCIENCE
ASSISTANT PROFESSOR IN HORTICULTURE

Applicants invited for a ten
stream position at the Assistant
fessor level, tenable June 1, 1979.
Ph D in Horticulture with a str
background in pomology and ph
ology, production and fruit culture
required The successful candidate
expected to participate in un
graduate courses and to develop
active research and teaching prog
at the graduate level Salary will be
the Assistant Professor scale depe
ing on experience Deadline for
ceipt of applications is April 15
when the position is filled A comp
curriculum vitae and the names
three referees should be sent to
W. F Grant, Chairman, Selec
Committee, Department of P
Science, Macdonald Campus, Mc
University, Ste Anne de Belle
Quebec H9X 1CO, Canada.

9551(A

POLYTECHNIC OF THE SOUTH BANK
DEPARTMENT OF PHYSICAL
SCIENCES AND TECHNOLOG
RESEARCH ASSISTANT

required to work with Dr D Ran
and Mr G. Beattie on the opti
photoelectric and allied properties
thin films in vacuo.
Salary scale, including Inner Lon
Allowance, £3,087 to £3,261
Apply by letter to Dr D. Rand
Dept. of Physical Sciences and T
nology, Polytechnic of the South B
Borough Road, London SE1 0AA

9547(A

THE UNIVERSITY OF LEEDS,
partment of Plant Sciences Grad
Technician in Electron Microsc
Applications are invited for the ab
post The person appointed will be
charge of the maintenance and p
aration work concerned with a rese
and teaching facility which incl
three transmission electron microsc
(a Siemens Elmiskop 1A, an A
EM6B and a Siemens 102), a scan
electron microscope (Cambridge 5
and ancillary equipment. Applic
should possess an H.N C. or equiva
qualification and have had substa
relevant experience. Salary on the
£3,654 to £4,365 a year. Applica
with the names of two referees
full details of qualifications and p
tical experience should be submitte
February 16, 1979 to The Secret
Department of Plant Sciences, Ba
Wing, The University, Leeds LS2

9548(

STDOCTORAL POSITION avail-
e immediately for the study of
lar immune responses (especially
)-immune phenomena) Experience
h techniques of cellular immun-
gy and tissue culture highly desir-
e. Salary $10,000 and up depending
experience. Send curriculum vitae
names of three references to: Dale
Gregerson, Ph D , Dept Ophthal-
ogy & Visual Science, Yale Uni-
ity School of Medicine, New
ven, Connecticut 06510 An Equal
portunity/Affirmative Action Em-
yer 9538(A)

UNIVERSITY OF MANCHESTER

CELLULAR IMMUNOLOGY GROUP

'ostdoctoral research assistant re-
red to collaborate on a project for
years studying the "Immunobiology
antigen-laden cells" Experience in
lular immunology or immunochemi-
techniques would be useful Initial
ary range p a : £3,883 to £4,382,
ng according to scale Requests for
ther details and applications (cur-
lum vitae and names of two ref-
es) should be sent to Dr Eric B
l, Department of Pathology, Uni-
ity of Manchester Medical School,
nchester M13 9PT. 9546(A)

PAPUA NEW GUINEA UNIVERSITY OF TECHNOLOGY

DEPARTMENT OF APPLIED PHYSICS

RESEARCH ASSISTANT

'he Department of Applied Physics
ites applications from suitable
sics graduates with interests in
physics or medical physics to
ry out research into the physical
perties of samples of blood with
cial reference to its rheological
perties.
'revious experience in this field of
earch is preferable, but not essen-
. Possibility of registering for a
her degree is available Other re-
rch interests of the Department are
the field of radiation and geophysics.
The research fellow will also be
uired to assist in tutorial classes
about eight hours a week.
'andidates should be single and be
pared to live in a University
dents' hostel They should be willing
take up the position by the end of
rch, 1979
alary between K7,000 to K8,000
annum
Applications should be addressed to
e Registrar, Papua New Guinea
iversity of Technology, P.O 793,
, Papua New Guinea and should
nprise a full curriculum vitae, age,
rital status, dependants, qualifica-
s, experience, and names and
dresses of three referees from whom
fidential enquiries can be made
Applications will close on *February*
1979 An additional copy of appli-
ion should be sent to Association
Commonwealth Universities (Appts),
Gordon Square, London WC1H
9481(A)

UNIVERSITY OF LONDON

INSTITUTE OF NEUROLOGY

MEDICAL LABORATORY SCIENTIFIC OFFICER

characterise normal and dystrophic
sma membranes using a combina-
n of immunological and biochemical
hniques Salary £3,261 plus £354
ndon weighting per annum. Send
ails of experience plus names of
referees to Dr F Walsh, Institute
Neurology, National Hospital,
een Square, London WC1N 3BG
9482(A)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE FOR RESEARCH ON ANIMAL DISEASES

A number of vacancies will shortly arise for

JUNIOR ANIMAL TECHNICIANS

in the Small Animal Section of the Institute

Preference will be given to candidates who have passed the Intermediate Examination of the I.A.T., but candidates currently engaged in or willing to pursue such a course of study will also be considered. Further study by day release up to F.I.A T. will be encouraged.

Appointments will be to Experimental Worker Grade, starting salary depending on experience.

Excellent working conditions in pleasant rural surroundings with staff restaurant and hostel accommodation for a single person, if required.

Non-contributory pension scheme.

Application Forms from: Secretary, Institute for Research on Animal Diseases, Compton, Nr. Newbury, Berks , quoting reference 401.

9516(A)

LINCOLN COLLEGE
(University College of Agriculture)
New Zealand

LECTURER IN CROP PHYSIOLOGY

Applications are invited for the position of Lecturer in the Department of Plant Science. Applicants should have qualifications and research experience in crop physiology, preferably combining a sound knowledge of physiological principles with personal experience of crop or pasture production Teaching at undergraduate and postgraduate levels will necessitate competence in such topics as photosynthesis, water relations, competition etc

Commencing salary according to qualifications and experience Present salary scale is: NZ$11,529 to $14,250. Travel and removal expenses are reimbursed up to specified limits New Zealand Government Superannuation is available

Initial enquiries may be made to the following now on study leave in England. Dr P. Jarvis, Plant Physiology Department, University of Sussex, or Dr W R Scott, Plant Breeding Institute, Cambridge.

Conditions of Appointment are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the College

Applications close on **April 16, 1979.**

9541(A)

UNIVERSITY OF OXFORD

RESEARCH OFFICERSHIPS IN NUCLEAR AND PARTICLE PHYSICS

The University proposes to appoint three Research Officers in the Department of Nuclear Physics for a period of three years, from October 1, 1979 or earlier if possible. Salary according to age on the University Lecturer scale (under review) £3,883 to £8,257 One post will be filled in experimental high energy physics and two in experimental structure physics. Further particulars may be obtained from the department

Applications (one typed copy) including a statement of qualifications, publications and the names of two referees, should reach Professor K W Allen, Department of Nuclear Physics, Keble Road, Oxford OX1 3RH by March 15, 1979 (Revised)

9518(A)

City of Glasgow District Council

MUSEUMS AND ART GALLERIES

Conservation Officer (Scientific)

Salary Scale: £3,279 to £4,773*

Applications are invited for a vacancy in a conservation science laboratory. Candidates should possess a good honours degree in physics, chemistry, metallurgy, materials science or a related subject. Museum or research experience would be advantageous.

The successful candidate will work closely with a large conservation staff and will be expected to develop methods of conservation treatment and apply scientific techniques into research into museum objects.

*Including Supplement

Superannuation, Sick Pay and Staff Life Assurance Schemes in operation. Removal expenses, legal fees and lodging and subsistence allowances payable in appropriate cases.

Appointment subject to medical fitness. Local authority salary and conditions apply.

Further details and an application form may be obtained from The Director, Art Gallery and Museum, Kelvingrove, Glasgow G3 8AG (Tel: 041-334 1134). Completed application forms to be returned by Friday, February 23, 1979.

PLEASE QUOTE REF. NA/6/79.

9489(A)

WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)

Chair of Pharmacology and Materia Medica

Applications are invited from suitably-qualified candidates in any branch of the sciences of pharmacology, including clinical pharmacology, for the above Chair in the University of Wales at the Welsh National School of Medicine, Heath Park, Cardiff. This post will become vacant following the retirement of Professor J.D P Graham and it is hoped that the successful candidate will be able to take up the appointment on October 1st 1979 or as soon as possible thereafter

The salary will be either within the scale for clinically-qualified Professors—currently up to a maximum of £12,C84 or that for non-clinical Professors (currently up to a maximum of £11,055) with participation in the Universities Superannuation Scheme

Further particulars for the post may be obtained from the **Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN** to whom applications should be submitted by 16th March, 1979.

9490(A)

If you are a graduate, age 26 or over, with a first or second class honours degree plus at least four years' relevant postgraduate experience and would like to make a career in research administration, a post of

Scientific Administrative Officer

in the Medical Research Council headquarters office in London should be of interest to you. Research experience in fields associated with medicine in the broadest sense and a research degree would be an advantage. Initially the successful candidate is likely to work in the Grants and Training Awards Section which is responsible for handling all aspects of the project grants, fellowships and studentships awarded by the Council.

The MRC is the main UK Government agency for the promotion of medical research and its headquarters office is responsible for the administration of an extensive programme of research which is carried out by directly supported staff in the Council's own establishments as well as by other research workers funded by MRC grants and other awards.

Salary will be in the range £5,619 to £7,363 per annum. In addition there is a pay supplement to compensate for contributions to the Council's pension scheme. Subject to satisfactory completion of a two years' probationary period, the appointment will be established; there is opportunity for promotion.

Application forms may be obtained by writing to or telephoning Miss M. Gale, Medical Research Council, 20 Park Crescent, London W1N 4AL (Tel. No. 636 5422 Ext. 35). Closing date for applications 26 February, 1979. Please quote P. 1002/39.

9529(A)

MRC
Medical Research Council

THE UNIVERSITY OF LEEDS
DEPARTMENT OF MEDICAL PHYSICS

Applications are invited for a temporary post of either **RESEARCH ASSISTANT** or **RESEARCH FELLOW** in the above department. Applicants should be graduates with a good honours degree and, for appointment at Research Fellow level, should possess a Ph.D. degree. The successful candidate will be required to take part in a study of bone mineralisation in patients with chronic rheumatoid arthritis. Mineralisation of the femur will be measured by gamma-ray absorptiometry and total body-calcium by in vivo neutron activation analysis and the person appointed will be responsible for calibrating the equipment, making measurements, collating data and improving methods of data processing. Candidates should have knowledge of radiation physics and experience of programming large and small computers. The appointment will be made for a fixed term of three years.

Initial salary in the range £3,384 to £4,133 on either the 1B scale (Research Assistant) (£3,384 to £4,882) or the IA scale (Research Fellow) (£3,883 to £6,555) for Research and Analogous Staff. Both scales are currently under review.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 92/2. Closing date for applications: March 2, 1979.

9474(A)

UNIVERSITY OF MALAWI
THE POLYTECHNIC

Applications are invited for the post of

LECTURER IN PHYSICS

in the DEPARTMENT OF MATHEMATICS AND SCIENCE. Applicants should possess a good degree in Physics. An interest in Mathematics would be an advantage. Previous teaching experience will be an advantage but is not essential. Appointee will be required to teach Physics, Mechanics and Electrical Science to diploma level in the field of engineering.

Salary scale: Lecturer K3,500 to K6,600 p.a. (£1 sterling to K1.62). The British Government may supplement salary in range £3,450 to £3,654 p.a. (sterling) for married appointee and £2,340 to £2,544 p.a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. If no British Government supplement is available, the University may pay an addition of between K1,320 to K1,656 p.a. for Lecturer grade (taxable in Malawi). Family passages; superannuation scheme; Gratuity 15% to 25%; biennial overseas leave; housing and various allowances. Detailed applications (2 copies) with curriculum vitae and naming three referees to be sent to Registrar, University of Malawi, PO Box 278, Zomba, Malawi by March 22, 1979. Applicants resident in UK should send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9480(A)

UNIVERSITY OF WOLLONGONG
Australia

PROFESSOR— MATHEMATICS (Ref. No. 919)

Applications are invited for the above-mentioned positon which has become vacant following the retirement of the Foundation Professor of Mathematics, Professor A. Keane. Applicants may have interests in any field of Mathematics, but the University would welcome, in particular, applications from those with an interest in Applied Statistics.

Applications close **March 23, 1979.**

PROFESSOR/ASSOCIATE PROFESSOR—PSYCHOLOGY

Following the appointment of the Foundation Professor of Psychology, Professor A. M. Clarke, to the position of Deputy Vice-Chancellor, the University invites applications for this vacancy which may be filled by either: a Professorial appointment, including Chairmanship of the Department, for a limited term of four years (Ref. No. 005) or (b) a tenurable appointment at Associate Professor level, with the appointee being required to serve as Chairman of Department for a minimum period of four years. (Ref. No. 991).

Applicants may have interests in any field of Psychology, but the University would welcome, in particular, applications from those with interests in empirically oriented research in community, clinical (counselling and abnormal) or social psychology or the psychology of human learning.

Applications close **March 9, 1979.**

Salary per annum: Professor $A33,061; Associate Professor $A27,916.

Subject to the consent of the University Council, professors may undertake a limited amount of higher consultative work. The University reserves the right to fill a Professorial or Associate Professorial vacancy by invitation.

For all positions, fares to Wollongong for the appointees and dependents are paid and a relocation allowance is payable. For a limited term appointment, the University would consider contributing to the cost of repatriation on conclusion of the appointment.

Applications should contain full details of qualifications, employment history, research interests, publications list, and the names and addresses of three referees, and should be forwarded to the Bursar, The University of Wollongong, Box 1144, P.O., Wollongong, N.S.W., 2500, Australia, from whom further information is available if required. Please quote the reference number of the position, and mark envelope "Confidential—Appointment". A copy of application should be sent to the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, from whom further information may also be obtained. 9539(A)

UNIVERSITY OF EDINBURGH
LECTURESHIP IN MICROBIOLOGY

Applications are invited for a newly established Lectureship in the Department of Microbiology. Applicants should have some experience or interest in applied microbiology, preferably in the area of agriculture or food.

Salary on scale £3,883 to £7,754 per annum, with superannuation benefits.

Further particulars may be obtained from the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, with whom applications by letter (five copies), including the names of three referees, should be lodged not later than March 7, 1979. Please quote Reference 1007.

9393(A)

THE UNIVERSITY OF HUL
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANT

Applicants are invited for a
chemist or biological chemist fr
October 1, 1979 for research un
the direction of Dr Colin Ratle
into the elucidation of the str
ture and physiological significance
iron-chelating compounds elabora
in the mycobacteria. Candidates sho
have or expected to have obtai
their Ph.D. before the starting d
The appointment, which will be
one year in the first instance, ren
able for a further two years, is s
ported by a grant from the SRC wh
also provides for technical assista
Salary will be on the lower part
the scale £3,883 to £4,882 (under
view).
Applications should give full det
of education, qualifications and
search experience, together with
names of two referees and should
sent by March 7, 1979 to Dr C. R
ledge, Department of Biochemis
University of Hull, Hull, HU6 7
from whom further details may
obtained. 9519(A

UNIVERSITY OF NEWCASTLE UPON TYN
DEPARTMENT OF GEOLOGY
LECTURER (SEDIMENTOLOGY)

Applications are invited for
lectureship (sedimentology) tena
from October 1, 1979. Applica
should have had research experie
within the field of clastic sedimen
logy. Experience in economic appl
tions of sedimentology, especially
this has been gained in industry, wo
be an advantage.
Salary will be at an appropr
point on the lecturer scale £3,883
£7,754 per annum according to a
qualifications and experience. Mem
ship of the appropriate Univer
superannuation scheme will be requi
Further particulars may be obtai
from the Registrar, The Univers
Newcastle upon Tyne NE1 7RU, w
whom applications (3 copies) toget
with the names and addresses of th
persons to whom reference may
made, should be lodged not later t
March 9, 1979. Please quote refere
N. 9505(A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF CHEMICAL PATHOLOGY
DIVISION OF STEROID ENDOCRINOLOGY

Applications are invited for th
temporary post of RESEARC
ASSISTANT for work on
project, supported by the Wellcon
Trust, to investigate the productic
of adrenocortical steroids
pregnant women. Applicants shou
possess a good honours degree in
science subject. Experience
paper chromotography would be a
advantage. There may be an oppo
tunity to register for a high
degree, and applications will
welcomed from new graduates
those who expect to graduate th
summer. The appointment will
made for a fixed period of up
two years.
Salary on the 1B scale f
Research and Analogous Sta
(£3,384 to £4,882) (under review)
Application forms and furth
particulars may be obtained fro
the Registrar, The Universit
Leeds LS2 9JT, quoting referen
number 85/1/D. Closing date f
applications: March 5, 1979.

9475(A)

UNIVERSITY OF CAMBRIDGE

LECTURESHIP

Applications are invited for a University Lecturer in Botany to take up appointment from October 1, 1979 or as soon as possible thereafter. The person appointed will be expected to provide teaching in the field of whole plant physiology and/or ecological plant physiology and to have research interests in the same areas of plant physiology. The appointment will be for three years in the first instance, with the possibility of reappointment to the retiring age. The salary is on an incremental scale rising from £5,367 to £8,257 per annum. Applications with the names of two referees should be sent by March 31, 1979 to the secretary of the Faculty Board of Biology 'A' Appointments Committee, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX, from whom further particulars may be obtained. 9531(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF FOOD SCIENCE

Applications are invited for a temporary post of RESEARCH FELLOW in the above Department. Applicants should have a Ph.D. degree, preferably in biochemistry or chemistry, and have an interest in applying their knowledge to the biochemistry of foods or to the chemistry of food additives. The successful applicant will have opportunities to cooperate with research institutes and to help with departmental teaching. The appointment will be made for a fixed period of three years.

Salary on the IA scale for Research and Analogous Staff (£3,883 to £6,555) (under review) according to age, qualifications and experience.

Interested persons who wish to discuss the post in more detail or visit the Department should contact Professor David S. Robinson (0532 31751, ext. 540).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 72/3/D. Closing date for applications, March 16, 1979.

9473(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of LECTURER in the above Department. The person appointed will participate in teaching biochemistry to science and to medical students. The Department provides good facilities and opportunities for research. The appointment will date from October 1, 1979.

Salary on the scale for lecturers: £3,883 to £7,754 (under review) according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 83/71/D. Closing date for applications, March 12, 1979.

9367(A)

UNIVERSITY OF ZAMBIA

Applications are invited for 2 posts of

SENIOR LECTURER/ LECTURER IN ORGANIC CHEMISTRY

in Department of Chemistry. Applicants should have a Ph.D. or equivalent and show evidence of research ability. The appointees would be expected to teach at all levels, from first-level to post-graduate courses and to participate in Departmental Research Programmes.

Salary scales: Senior Lecturer K7,608 to K8,016 p.m. Lecturer K5,904 to K7,608 p.a. (£1 serling=K1.56). The British Government may supplement salary in range £2,922 to £4,350 p.a. (sterling) for married appointees or £930 to £1,968 p.a. for single appointees (reviewed annually and normally free of all tax) and provide children's education allowance; superannuation; medical aid scheme; regular overseas leave; three year contract. Detailed application (2 copies) with curriculum vitae and naming 3 referees to be sent to Regisrar, University of Zambia, PO Box 2379, Lusaka, Zambia by March 16, 1979. Applicants resident in the UK should send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9483(A)

CHARING CROSS HOSPITAL

BASIC GRADE BIOCHEMIST

Applications are invited from honours graduates for the above post in the Department of Chemical Pathology.

This is a busy, highly automated department with computerised data handling and the successful applicant will be expected to participate in all aspects of the routine work.

Previous experience and/or higher qualifications in clinical chemistry would be an advantage, but are not essential. Study for further qualifications will be encouraged.

Informal enquiries and requests to visit the department should be directed to Dr O. Swale (Tel: (01) 748 2040 ext 2779 or 2772). Whitley Council conditions of employment apply. Salary scale £3840—£4629 per annum inclusive. Application forms from District Personnel Department, Charing Cross Hospital, Fulham Palace Road, Hammersmith, London W6 Tel: (01) 748 2040 ext 2992/3021.

Closing date for receipt of applications 1 March 1979. 9499(A)

MEDICAL RESEARCH COUNCIL

RADIOBIOLOGY UNIT

A SCIENTIST

is required to join a team studying the distribution and effects of radioactive materials in animals. The appointee will initially be concerned with studies on the distribution and dosimetry of radionuclides in tissues but other interests in radiobiology will be encouraged. Applicants should preferably be well qualified in physics or biology and have at least 3 years postgraduate experience in research. Experience in the use of computers would be an advantage.

Salary in accordance with age and experience on M.R.C. Grade II/I, £3,883 to £7,754 (as at October 1, 1978, with negotiations in progress).

Enquiries or application with curriculum vitae and names of two referees to **Dr J. Vennart, Director, M.R.C. Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD,** by March 9, 1979.

9591(A)

MRC
Medical Research Council

FELLOWSHIPS

RESEARCH FELLOWSHIP

Firemen's Health Research – Monitoring of ECG and other studies

Scientific Advisory Branch
Horseferry House, London, SW1

Senior/Junior Research Fellow

The successful candidate will be appointed to help carry out measurements of heart rate and other physiological functions as part of a work programme investigating stress in firemen's work. The officer will help in planning and developing a study programme for ECG measurement on groups of active firemen and for implementing and maintaining the measurement procedures.

The officer will form part of a small team working on a number of aspects of the health of firemen and will be responsible to a Senior Scientific Officer concerned with the research planning of the team.

The applicants should have an honours degree or equivalent in human physiology with some practical experience in the use of physiological apparatus and the measurement of cardiac and respiratory function. A background in physics and chemistry would be an advantage.

Some travel will be necessary.

The fellowship will be tenable for two years and remuneration will be on the scale £3695-£4580 for Junior Research Fellow and £5092-£6216 for Senior Research Fellow.

Apply in writing by 16th March, 1979 to Miss S. M. Swinborne, Room 203, Whittington House, 19-30 Alfred Place, London WC1E 7EA.

9477(E)

Home Office

FELLOWSHIPS—continued

M.R.C. CLINICAL RESEARCH CENTRE

(Northwick Park Hospital)
WATFORD ROAD, HARROW
MIDDX. HA1 3UJ

M.R.C. TRAINING FELLOWSHIPS

Applications are invited from suitably qualified graduates to work in any one of the following areas:

1. Immunochemical analyses of serum immune complexes, in particular those occurring in E.B. virus infections (infections mononucleosis) and mycoplasma pneumoniae infections. It is also planned to investigate the possible role of auto-anti-idiotypes in the control of the anti-host tissue antibodies (e.g. crythrocyte autoantibodies) in these infections.
2. The neuropathology of mental disease.
3. Immunopathology of C.N.S. receptors.
4. Applications of immunological methods to clinical problems.

Eligibility requirements: From post-registration up to senior registrar or equivalent academic levels PHD/D.PHL. or MSC plus at least 3 years postgraduate experience.

Tenure: Up to three years.

Stipend: On the current NHS medical (House Office to Senior Registrar) or current relevant academic scale.

The successful applicants will be required to apply for a medical research training fellowship through the normal annual competition for these awards.

Please send curriculum vitae to Mrs J. Tucker-Bull by **FEBRUARY 16th.**

9545(E)

UNIVERSITY OF SASKATCHEWAN

GERHARD HERZBERG FELLOWSHIPS FOR GRADUATE STUDY

In honours of Dr Gerhard Herzberg, 1971 Nobel Laureate in Chemistry, and to commemorate his service to Saskatchewan, the University of Saskatchewan offers a graduate fellowship valued at $7,000 per annum, renewable for a second year. Herzberg Fellows are eligible for a part-time laboratory demonstratorship which provides additional remuneration up to $2,000 per annum.

The fellowship is tenable in the Department of Physics or the Department of Chemistry and Chemical Engineering by a candidate for a graduate degree in spectroscopy or a related field.

Letters of application with appropriate documentation and the names of two referees should be addressed to the Dean, College of Graduate Studies and Research, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0. Applications for Fellowships for the 1979-80 academic year should be made prior to March 15, 1979.

9485(E)

RESEARCH FELLOWSHIP

IMPERIAL CANCER RESEARCH FUND

We have a vacancy for a postdoctoral Research Fellow to join a working group on markers of neoplastic transformation during ageing and carcinogenesis *in vitro*, in epithelial cell systems. The specific collaborative project will be concerned with isolation of hybrids, using irreversible biochemical inhibitors, identification of transformed characters and the association of these characters to DNA and chromosome changes. Special experience in cell culture or cytogenetic techniques required.

Appointment will be for three years. Salary according to qualifications and experience.

Further information from Dr L. M. Franks (01-242 0200, ext. 211). Applications with curriculum vitae and names of two referees should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX.

9555(E)

FELLOWSHIPS—continued

LANCHESTER POLYTECHNIC

Coventry

Department of Biological Sciences

Postdoctoral Research Fellowship

(Tenable for up to 3 years from March 1, 1979)
£4,101 - £6,558

THE JOB · The project is funded jointly by the SRC and ICI and involves the development of a method for the *in vitro* culture of perifused hepatocytes supported in a 3-dimensional matrix and its application to toxicological studies

REQUIRED Experience in animal cell culture and biochemistry Knowledge of cytological procedures would be an advantage

For further Information applicants may contact Dr Sturdee or Dr Crisp by telephoning Coventry (0203) 24166

Faculty of Applied Science

Research Assistant in Cardiac Pacing

(Tenable for 1 year from March 1, 1979)
£3,192 - £3,330

THE JOB · A statistical survey of patients with artificial cardiac pacemakers is required to attempt to identify those factors which cause a rise of stimulation threshold in certain patients The required collection of data will involve close collaboration with cardiac pacing centres in a number of hospitals

REQUIRED · Applicants should have either an appropriate Honours Degree, or an appropriate HNC plus relevant experience

For further Information applicants may contact Dr. Economides or Dr Duckers by telephoning Coventry (0203) 24166

For application forms and further particulars for the above posts please apply in writing enclosing a foolscap stamped and addressed envelope to the Personnel Officer, Lanchester Polytechnic, Priory Street, Coventry CV1 5FB, applications returnable by February 19, 1979 8404(E)

RESEARCH FELLOWSHIP

Imperial Cancer Research Fund Laboratories,
Mill Hill, NW7

A postdoctoral Fellow is required to join a group working on the genetic and biochemical analysis of certain heat activated loci in Drosophila melanogaster, using cloned DNA fragments to investigate their organisation and expression. Experience in molecular biology desirable.

Appointment will be for three years. Salary with entry according to qualifications and experience within range £5,332 to £6,530.

Further information from Dr Ish-Horowicz (01-959 3236) Applications with curriculum vitae and names of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields WC2A 3PX by February 24, 1979.

9428(E)

History of Medicine Fellowships

The Wellcome Trust provides grants for research in the history of medicine and maintains Wellcome Units for the History of Medicine in the Universities of London, Oxford and Cambridge, the Wellcome Institute for the History of Medicine in London, and also provides support for a small Unit at Edinburgh University.

Applications are invited for research fellowships of up to three years, to be held in the Wellcome Units or the Wellcome Institute. Applicants must hold a doctoral degree or an equivalent qualification and have a background in history, science or medicine. Applicants should write in the first instance, outlining their research proposals and enclosing their *curriculum vitae*, to the Director of the Unit in which they wish to work. The addresses of the Units are as follows, and letters should arrive **not later than 1st March:**

**The Wellcome Institute for the History of Medicine,
183 Euston Road, London NW1 2BP.**

Wellcome Unit for the History of Medicine, 47 Banbury Road, Oxford OX2 6PE.

Unit of History of Medicine, University College London, Gower Street, London WC1E 6BT.

Wellcome Unit for the History of Medicine, Free School Lane, Cambridge CB2 3RH.

The Wellcome Lecturer in the History of Medicine, Department of History, University of Edinburgh, William Robertson Building, George Square, Edinburgh EH8 9JY.

If the Director of the academic unit concerned agrees to support the proposal, it will be forwarded to the Wellcome Trust for a decision on whether a fellowship can be awarded. Applicants should write to their first choice only. If unsuccessful, the application may be passed to other Units if the candidate requests it.

THE WELLCOME TRUST

1 Park Square West, London NW1 4LJ 9508(E)

APPLICATIONS
are invited for

CHARLES A. REVSON FELLOWSHIPS
at
THE HEBREW UNIVERSITY OF JERUSALEM

The Fellowships are being offered in the Faculties of Agriculture, Science and Mathematics, Medicine and Dental Medicine (in the pre-clinical areas) and the Schools of Pharmacy and Applied Science and Technology

The Fellowships, which will be awarded to outstanding scholars for a period of three to four years, are mainly at the *rank of lecturer* with a limited number also available at the rank of senior lecturer or above. Fellows will be required to carry out teaching and research in one of the above fields

Applicants should be residents of Israel or persons intending to settle in Israel.

Appointments will be made as from July 1979 and not later than October 1980 Academically suitable Charles A. Revson Fellows will be offered posts as regular faculty members at the Hebrew University of Jerusalem on completion of their Fellowships

Applications accompanied by a full curriculum vitae, list of publications, reprints of three recent publications, names of referees and date of availability, should reach the Academic Secretary, The Hebrew University of Jerusalem, Israel, by March 15, 1979. 9552(E)

FELLOWSHIPS—continued

DEPARTMENT OF
VETERINARY PHYSIOLOGY

UNIVERSITY OF EDINBURGH

S.R.C. funded postdoctoral research fellowship to join a group working on somatosensory mechanisms in the mammalian spinal cord. Tenable for three years, starting salary scale point 3 on range 1A national research salary scale. Candidates should be experienced in neurophysiological techniques. Experience in laboratory-oriented computing will be an advantage.

For further information contact Professor A. Iggo, Department of Veterinary Physiology, Royal (Dick) School of Veterinary Studies, Edinburgh EH9 1QH.

Applications, by letter (two copies), giving the names of two referees, should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, not later than March 2, 1979. Please quote reference 5003.

9532(E)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF
BIOCHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP/ ASSISTANTSHIP

(Re-advertisement)

Applications are invited for an S.R.C. Research Fellowship to determine the amino acid sequence of pyruvate kinase. The project is a collaborative one with the protein crystallography group at the University of Bristol, and will lead to a detailed description of the molecular structure of pyruvate kinase.

The successful applicant will work, in collaboration with a predoctoral research assistant already in post, with Dr L. A. Fothergill's research group which is currently studying the structures of four other glycolytic enzymes.

The appointment is available for a period of up to three years. Salary within Range 1A, £3,883 to £4,882 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by March 2, 1979.

9576(A)

New Zealand

UNIVERSITY OF CANTERBURY

Christchurch

Applications are invited for a University

POSTDOCTORAL FELLOWSHIP

in the following Department. Applicants should possess a Ph.D. or equivalent degree, preferably followed by some research experience. The appointee may be asked to undertake a limited amount of teaching in addition to contributing to the current research programme in their particular field:

BOTANY: The fields within which the Fellowship may be held are: Reproductive biology of flowering plants; Soil biomass studies; Fine structure and taxonomy of marine nanno plankton.

Applications close on **March 31, 1979.**

The emolument will be NZ$8,500 per annum. Fellowships are tenable for one year.

Further particulars and Conditions of Appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

9592(E)

MONASH UNIVERSITY

Melbourne, Australia

POSTDOCTORAL FELLOWSHIP IN ECOLOGY-ANIMAL/PLANT INTERACTIONS

The University is offering a 2 year appointment to initiate studies in a local, natural ecosystem, with primary emphasis on the relationships between fauna and flora. Examples of possible fields of study are ant/eucalypt interactions, litter decomposition, plant/herbivore systems, etc. The appointment is jointly in the Department of Botany and Zoology. Applications should include a general statement of the proposed research. Salary: Junior Research Fellow $A13,083 p.a. Travelling and removal allowance. Assistance with temporary housing. Applicants should quote reference No. 43065 Closing date:: **February 28, 1979.**

Applications in duplicate to the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria, Australia 3168 giving age, qualifications, experience and names and addresses of three referees.

9540(E)

THE UNIVERSITY OF HULL

DEPARTMENT OF
APPLIED PHYSICS/PHYSICS

Applications are invited for three research fellowships, which are immediately available, for a period of up to three years.

Department of Applied Physics

1. A research fellow is required to measure the frequency characteristics of the current generation of pulsed CO_2 lasers and to devise ways of improving their frequency stability using techniques such as hybridisation and injection locking.
2. A research fellow is required to work on the use of timable multi-atmosphere, molecular gas lasers for the remote sensing of trace gases in the atmosphere.

Department of Applied Physics/
Physics

3. A research fellow is required to investigate the use of ion implantaiont for fabricating fast, photovoltaic, infrared detectors in materials such as HgCdTe.

These fellowships are suitable for graduates with at least three years' research experience in the fields of optics and quantum electronics (for 1 and 2) and solid state physics (for 3).

Salary scale: £3,883 to £6,555 per annum (under review).

Applications should give full details of education, qualifications and research experience, together with the names of two referees, and should be sent by February 22, 1979 to Dr. E. L. Thomas, Department of Applied Physics, The University of Hull, Hull HU6 7RX from whom further details may be obtained. 9520(E)

UNIVERSITY OF BRISTOL

SCHOOL OF CHEMISTRY

POSTDOCTORAL FELLOWSHIP IN LASER SPECTROSCOPY AND KINETICS

Applications are invited for a Postdoctoral Research Fellowship to work on the electronic spectroscopy and state to state kinetics of free radicals. The project will make use of both continuous wave and pulsed dye lasers and an inert gas halide laser, and will be oriented to problems of astrophysical importance. The post is S.R.C. funded, and the initial salary will be in the range £3,883 to £4,382 p.a. (scale under review). Applicants should send a curriculum vitae and the names of two referees to Professor R. N. Dixon, School of Chemistry, The University, Bristol BS8 1TS, U.K.

9533(E)

THE ANIMAL VIRUS RESEARCH INSTITUTE

Pirbright,
Woking, Surrey GU24 0NF

Applications are invited for a

WELLCOME RESEARCH FELLOWSHIP

tenable for 3 years. The successful candidate will investigate the physical and chemical structure of picornaviruses with the aim of ascertaining those factors which determine the stability of the capsid structure of foot-and-mouth disease virus. Applicants should preferably hold a Ph.D. degree and have had previous experience in virology and protein chemistry but students graduating this year are invited to apply. Opportunity will be provided to study for a higher degree.

Tax free stipend £4,000 p.a. for Ph.D.; £3,000 p.a. for new graduate.

Applications to the Secretary from whom further details can be obtained.

Closing date: March 15, 1979.

9595(E)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF
CHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from graduates in Chemistry or Physics to work wih Dr J L Duncan and Dr D C McKean on Infra-red Spectroscopy. The successful applicant will undertake research on a variety of topics using a computer controlled, high resolution Fourier transform spectrometer. Previous interactive experience wih computers, and an ability to develop software and undertake electronic maintenance is desirable.

The post is funded by the Science Research Council and is available from April 1979. Appointment will be for one year in the first instance, with possible extension up to a maximum of three years.

Commencing salary within Range 1A, £3,883 to £4,382 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen with whom applications (two copies) should be lodged by February 23, 1979.

9506(E)

ASSISTANTSHIPS

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF INORGANIC,
PHYSICAL AND INDUSTRIAL
CHEMISTRY

THEORY OF VIBRATIONAL PHENOMENA IN ADSORBATES

Applications are invited for an S.R.C. postdoctoral Senior Research Assistantship at an initial salary of between £3,883 and £4,382 per annum, with superannuation benefits. The post is tenable for one year in the first instance, and may be renewed for a further year. The project is the theoretical study of vibrational phenomena in adsorbates including elec energy loss spectroscopy, with Professor T. B. Grimley. The successful applicant will join an active group involved in the theoretical study of molecular and electronic processes on solid surfaces.

Applications stating age, academic qualifications and experience, together with the names of two referees should be received not later than March 5, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: 479/N. 9522(P)

UNIVERSITY OF CALGARY

GRADUATE ASSISTANTSHIPS

(Teaching and Research)

Available for students proceeding the M.Sc. and Ph.D. degrees in a branches of CHEMISTRY and BIO CHEMISTRY. The assistantships a valued at $7,000 to $8,000 p.a. a include remission of program fee Travel allowance also available.

For further information write to:

Department of Chemistry
Chairman of Graduate Studies
UNIVERSITY OF CALGARY
Calgary, Alberta, Canada
T2N 1N4

Please indicate field of interest.

9462(P)

UNIVERSITY OF HULL

DEPARTMENT OF
APPLIED PHYSICS

RESEARCH ASSISTANTSHI NON DESTRUCTIVE TESTING

Applications are invited for a r search assistantship tenable for a perio of up to two years in the first instanc with the possibiliy of renewal. Th research assistant will be involved i the study of magnetic particle inspec tion testing and ultrasonic testing of wide variety of industrial component in collaboration with the Health an Safety Executive, Nuclear Inspecto rate.

Salary scale: £3,384 to £4,882 (unde review) together with USS benefits.

Applications, which should includ the names of two referees, should b sent as soon as possible, to Dr S Palmer, Department of Applied Ph sics, The University of Hull, Hul HU6 7RX from whom further part culars may be obtained.

9465(P)

THE HATFIELD POLYTECHNIC

PO Box 109,
Hatfield, Herts. AL10 9AB

SCHOOL OF NATURAL
SCIENCES

RESEARCH ASSISTANTSHIPS

POST 1 MAMMALIAN PHYSIOLOGY Ref: 167

There is an immediate vacancy for a suitably qualified graduate to work for a higher degree on non-adrenergic and non-cholinergic transmission on the rat gastric corpus. The post is tenable for two years in the first instance with possibility of extension for a third year.

For further details apply to Dr K. Wilson, Biological Sciences, Telephone Hatfield 68100, extension 483.

POST 2 ASTRONOMY AND EARTH SCIENCES GROUP Ref: 168

Applications are invited from graduates with an honours degree in geology to work on a contract to investigate rock weathering sponsored by the Overseas Unit of the Transport and Road Research Laboratory. An interest in applied petrology would be an advantage.

The appointment will be for an initial period of two years with the possibility of an extension for a further year. The person appointed would be expected to register for a higher degree.

The successful candidate will be expected to take up the post on April 1, 1979, or as soon as possible thereafter.

Informal enquiries to Dr D. Cawsey, Hatfield 68100 extension 283, or write or telephone the Staffing Office, extension 309.

Salary scale for both posts (which are superannuable): £2,613 to £2,700 to £2,787 per annum plus £159 local weighting.

Closing date: February 26, 1979.

9530(P)

ASSISTANTSHIPS—cont.

PHILIPPS-UNIVERSITY MARBURG

DEPARTMENT OF PHARMACOLOGY

SCHOOL OF MEDICINE AND HUMAN BIOLOGY

Applications are invited for a two-year **POSTDOCTORAL RESEARCH ASSISTANTSHIP** (DFG, BAT II) for work with Dr G. F. Fuhrmann on problems of yeast plasma membrane vesicles—structure and function. Applicants with interest in membrane research and pharmacology should write to: Professor Dr G. F. Fuhrmann, Department of Pharmacology, Philipps-University Marburg, Lahnberge, D-3550 Marburg, Germany. 9584(P)

STUDENTSHIPS

GLASSHOUSE CROPS RESEARCH INSTITUTE

invites applications for an

AGRICULTURAL RESEARCH COUNCIL STUDENTSHIP

enable at the Institute for three years or work on the potential of the insect-pathogenic muscardine fungi to control hrips and leafhoppers. Minimum qualification first or upper second class honours degree in biological subjects with emphasis on microbiology, particularly mycology. Interest in biological pest control desirable.

Application forms and further particulars obtainable from Secretary, G.C.R.I., Worthing Road, Rustington, Littlehampton, Sussex BN16 3PU. Closing date for applications February 26. 9549(F)

AGRICULTURAL RESEARCH COUNCIL STUDENTSHIP

Applications are invited for an A.R.C. postgraduate studentship tenable at the Meat Research Institute for research into the viscoelastic properties of semi-solid foodstuffs.

The work will involve a study of he transmission of mechanical pulses hrough foods and computer analysis of transient sresses generated with an Instron materials tester.

The project is expected to form the basis for a Ph.D. thesis at the University of Bristol and the appointment will commence in October 1979.

Candidates should possess a First or Upper Second Class degree in Physics or a related subject.

Further particulars of the award and allowances together with application forms may be obtained from the Personnel Officer, Agricultural Research Council, Meat Research Institute, Langford, Bristol BS18 7DY. 9484(F)

PLANT BREEDING INSTITUTE

Maris Lane, Trumpington, Cambridge CB2 2LQ

CYTOGENETICS/PHYSIOLOGY DEPARTMENTS

POSTGRADUATE STUDENTSHIP

Applications are invited for an Agricultural Research Council postgraduate studentship leading to a Ph.D. in the University of Cambridge October 1979, to work on the genetics and biochemistry of protein components relating to bread quality in wheat. The student required should hold a first or upper second class honours degree in a biological science in which genetics and biochemistry feature strongly.

Remuneration will be in accordance with the Agricultural Research Council Regulations Governing Postgraduate Awards.

Applications together with a curriculum vitae and the names and addresses of three referees should be sent to the Assistant Secretary (Establishment), quoting reference CYT/48, as soon as possible. 9493(F)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY

RESEARCH STUDENTSHIPS

Studentships, commencing in October for postgraduate work leading to a higher degree in London University, may be available later in the year for research on several topics, including the structure and function of membranes. Enquiries from interested final year undergraduates should be addressed to Professor J.A. Lucy, Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP. 9471(F)

HAMILTON KERR INSTITUTE

University of Cambridge

STUDENTSHIPS IN THE CONSERVATION OF EASEL PAINTINGS

Applications are invited from men and women preferably between the ages of 20 and 25 for one studentship of three years duration starting September 1979 with an optional fourth year internship in an approved context. Admission for a shorter period will be considered from partly trained students transferring from their conservation courses.

The Institute is fully equipped with studios, a workshop and scientific laboratories. There is a technical and art-historical library; the libraries and scientific facilities of the University (including the Fitzwilliam Museum) are also available. The students play an active part in the Institute, and there are also courses in theoretical and academic instruction. Students are normally eligible for membership of a College.

A student should normally have a first degree or equivalent qualification or experience in relevant subjects such as The Sciences, Medicine, or Fine Arts. Evidence of manual and artistic skills is essential.

Fees chargeable by the Institute are £750 p.a. for UK and £850 p.a. for overseas residents. A successful UK applicant may be eligible for a state grant for which the Institute itself would apply to the Department of Education and Science. In the case of a state grant being awarded the fees are payable by the DES.

Application forms together with the Institute's prospectus may be obtained from:

The Secretary
Hamilton Kerr Institute
Whittlesford
Cambridge CB2 4NE

The closing date for receipt of candidates application forms is March 23, 1979. 9491(F)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF FIBRE SCIENCE

RESEARCH STUDENTSHIPS

Applications are invited from suitably qualified science or engineering graduates for two studentships. One for work on the handle and texture of fabrics would suit a candidate with a physics/mathematics background. The other dealing with fibre structure/property relationships would suit a chemist or materials scientist.

S.R.C. scales will apply and successful applicants will be expected to register for a higher degree.

Further information and application forms can be obtained from:

Dr H. M. Elder,
Chairman of Department,
Department of Fibre Science,
University of Strathclyde,
George Street, Glasgow.

9572(F)

INSTITUTE FOR RESEARCH ON ANIMAL DISEASES

A.R.C. POSTGRADUATE STUDENTSHIPS

Applications are invited from students interested in research in the field of animal diseases.

Candidates should have studied biological science, with particular interests in biochemistry, physiology, bacteriology or zoology and will be required to register for a higher degree, usually at the University of Reading.

Candidates should have, or expect soon to obtain, a 1st or Upper Second class honours degree in a relevant subject.

The Institute is situated in a pleasant rural area with a Staff Restaurant and good recreational facilities. If required, accommodation for a single person could be provided in the Institute's Hostel.

The Agricultural Research Council's pension scheme is non-contributory.

Application Forms from: Secretary, Agricultural Research Council, Institute for Research on Animal Diseases, Compton, Nr. Newbury, Berks., quoting Ref. 400. 9517(F)

MISCELLANEOUS

F.D.A. FOREIGN BODY CARCINOGENESIS STUDY

The Food and Drug Administration plans to conduct a study to distinguish the chemical carcinogenic potential of implant materials from their ability to induce tumours as a result of their surface characteristics (foreign body carcinogenesis). The in vivo study will be for a twenty-four (24) month period using rats or any other appropriate animal model. The contractor should have experience in carcinogenesis studies, particularly those involving modern cytogenetics, pathology, hematology and tissue culture techniques. Requests for RFP No. 223-79-5061 should be directed to Beverly Lott, Department of Health, Education and Welfare, Public Health Service, Food and Drug Administration, HFA-514, 5600 Fishers Lane, Rockville, MD, 20857. 9583(J)

GRANTS & SCHOLARSHIPS

POSTGRADUATE RESEARCH

DEPARTMENT OF BIOLOGY

PRINCETON UNIVERSITY

N.I.H. postdoctoral training grant positions for recent Ph.D.'s in the areas of biochemical and genetic aspects of cell differentiation (Drs T. Cline, E. Cox, A. Newton, P. Schedl), cell biology and development (Drs J. Bonner, E. Cohen, M. Steinberg, G. Witman), and behavioural and population genetics (Drs J. Endler, J. Gould, R. May, W. Quinn) are available beginning summer, 1979. Applications should include curriculum vitae and summary of research interest.

Closing date April 2, 1979. Inquiries or applications should be sent to Dr E. C. Cox, Chairman, Department of Biology, Princeton University, Princeton, N.J. 08540.

Princeton University is an Equal Opportunity/Affirmative Action Employer. 9388(H)

THE NATIONAL KIDNEY RESEARCH FUND invites applications for grants for renal research at units within the United Kingdom. Application forms are available from The Secretary, 184B Station Road, Harrow, Middlesex HA1 2RH (01-863 4469). 9408(H)

FOR SALE & WANTED

FOR SALE: Annual Reports of Progress of Applied Chemistry, 1941–1973 plus various other books on water (new and second-hand). Offers to: Box No. 9588(L), Nature, 3 Dyers Buildings, Holborn EC1N 2NR. 9588(L)

SYMPOSIA

PROSTAGLANDINS AND INFLAMMATION

An International Research Symposium

will be held at King's College Hospital Medical School, London, on Thursday and Friday, August 2 and 3, 1979.

Further details and registration form from:

Dr A. W. Ford-Hutchinson,
Biochemical Pharmacology
Research Unit,
King's College Hospital
Medical School,
Denmark Hill,
London SE5 8RX.
Tel: 01-274 6222, Ext. 2026.

9478(M)

COURSES

UNIVERSITY OF ESSEX

DEPARTMENT OF BIOLOGY

M.Sc. COURSE: GENE TECHNOLOGY IN MICROORGANISMS

Applications are invited for the above 1 year course for October 1979. A limited number of studentships may be available. The course is designed to acquaint the student with the techniques of classical microbial genetics, and in vitro recombinant DNA techniques. Enquiries should be addressed to The Chairman, University of Essex, Department of Biology, Wivenhoe Park, Colchester, CO4 3SQ. 9510(D)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

CENTRE FOR ENVIRONMENTAL TECHNOLOGY

M.Sc. IN ENVIRONMENTAL TECHNOLOGY 1979/80 ENERGY POLICY

One of the specialist options being offered as part of the one year Postgraduate and Post Experience Course at the interdepartmental Centre for Environmental Technology at Imperial College.

Course starts with a series of core subjects covering physical environment, applied ecology, urban technology, mineral resources, energy, environmental economics and policy, operations research, computing, and sources and choices of technology.

In the second term students specialise in one of six options. The Energy Policy option is designed to provide an understanding of the technological and political priorities of the energy industries and the sector as a whole; emphasis is placed on the relationship between the technology and the other considerations.

The remainder of the year is devoted to a research project. Projects in conjunction with outside bodies are welcomed.

Course is open to science and engineering graduates and other suitably qualified students, both from UK and overseas. Post experience students may divide the course between two academic years.

N.E.R.C., S.R.C. and S.S.R.C. have approved the course for tenure of their advanced course studentships.

Closing date for applications: April 1, 1979.

Further particulars from the Registrar, Imperial College of Science and Technology, London SW7 2AZ. 9512(D)

NATO/EMBO

INTERNATIONAL SUMMER SCHOOL on

NUCLEIC ACID PROTEIN RECOGNITION

Aug. 20 - Sept. 2, 1979 - Island of Spetsai, Greece

Lectures and discussions in English will cover the following topics:

1) Techniques for studying nucleic acid protein recognition.
2) Organization of genes in DNA.
3) DNA protein interactions (nucleosomes and proteins involved in RNA transcription).
4) RNA protein interactions (viruses and proteins involved in translation).
5) Theoretical aspects of nucleic acid protein recognition.

The lecturers will include:

H. Bujard (Germany), **C.R. Cantor** (USA), **P. Chambon** (France), **B.F.C. Clark** (Denmark), **B.S. Cooperman** (USA), **H. Delius** (Germany), **J.P. Ebel** (France), **W. Gilbert** (USA), **D. Glover** (UK), **F. Gros** (France), **M. Grunberg-Manago** (France), **C. Hélène** (France), **J. Hershey** (USA), **A. Klug** (UK), **P. Little** (UK), **A. Rich** (USA), **R.J. Roberts** (USA), **V. Sgaramella** (Italy), **T. Steitz** (USA), **P.H. Von Hippel** (USA), **C. Weissmann** (Switzerland), **H. Zachau** (Germany).

The Summer School will be suitable for predoctoral students and for post-doctoral workers in related fields.

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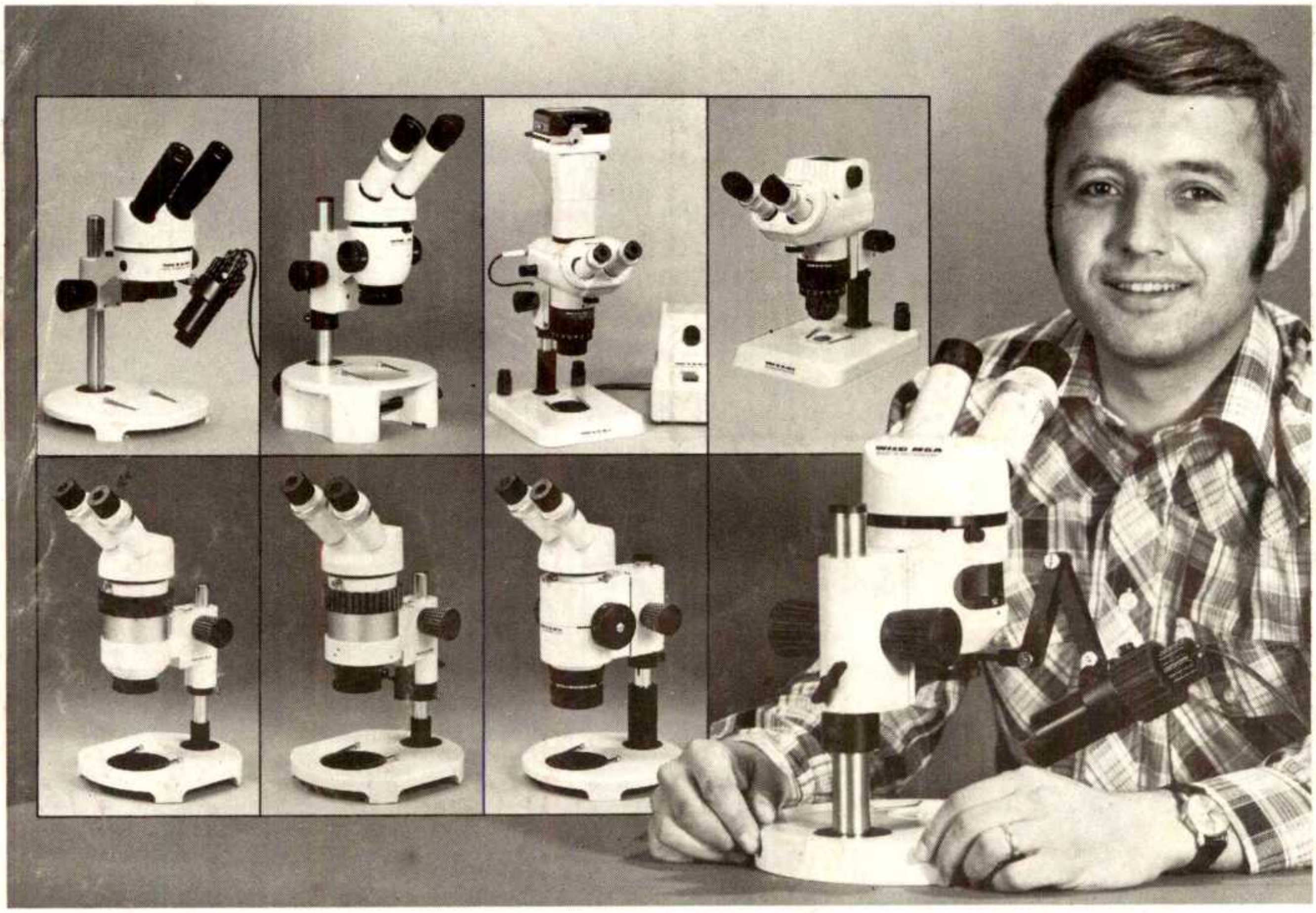

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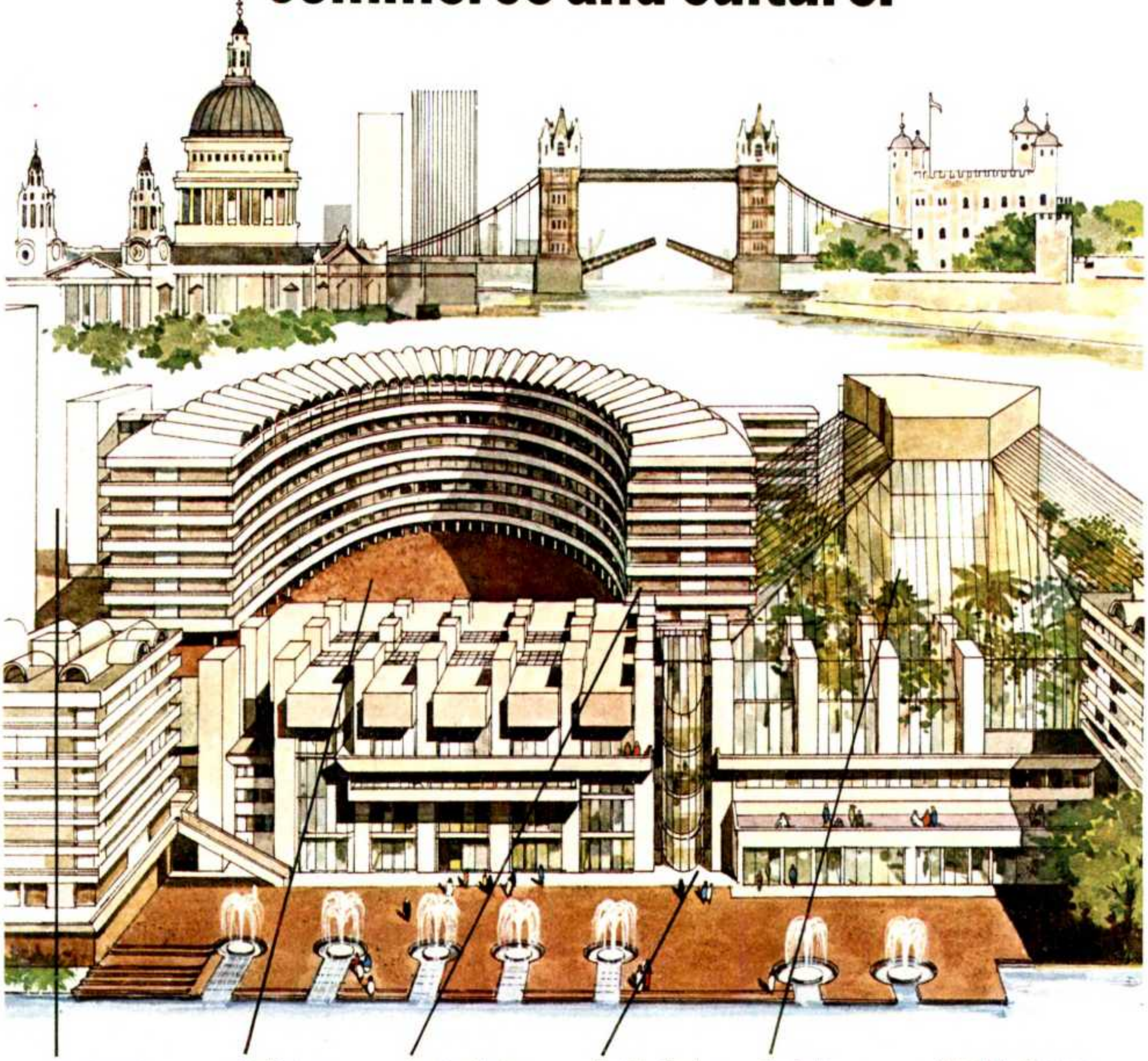

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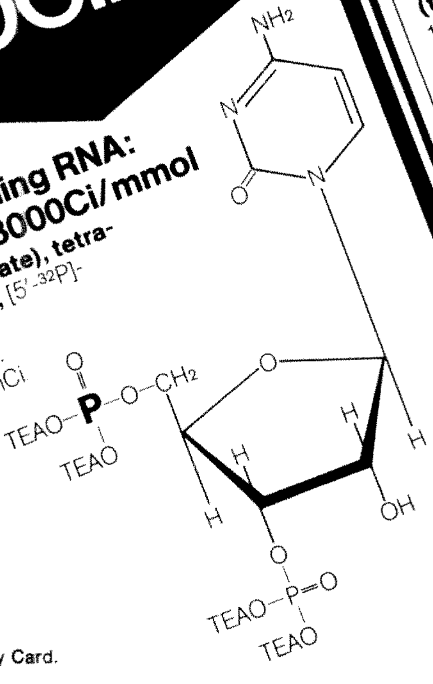

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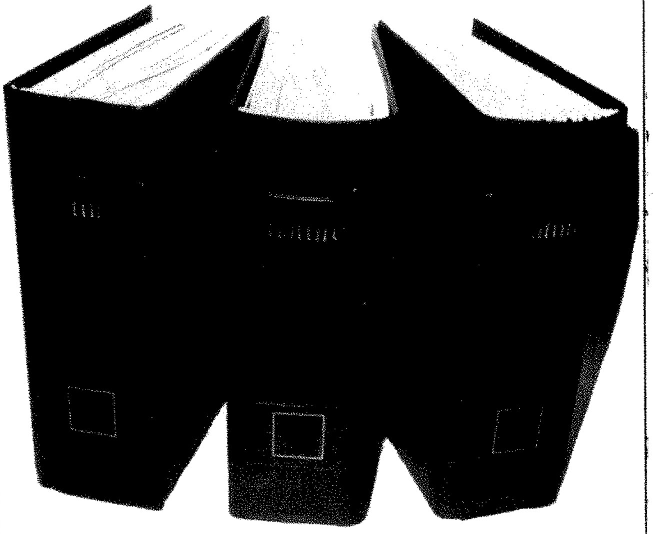

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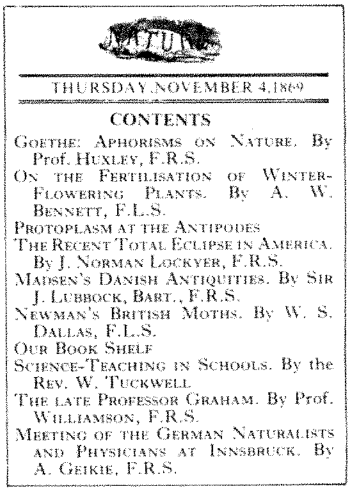

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nature 15 February 1979

After geodynamics, what?

THE earth sciences depend very largely on international collaboration; so it is no surprise that there has been a succession of projects aimed at nurturing the free flow of data and ideas across national boundaries. International Polar Years, and the International Geophysical Year of 1957 led in the 1960s to the Upper Mantle Project and in the 1970s to the Geodynamics Project, which closes at the end of this year. Questions are inevitably being asked now about a possible successor to these programmes, and in Zurich last week, under the aegis of the International Union of Geological Sciences (IUGS), a wide variety of earth scientists gathered to review the state of their specialisations and to mull over a proposal for collaboration in the 1980s.

What does a low-budget international scientific programme achieve? In some respects very little. It offers no research funds Most scientists in their national context are unlikely ever to read the rationale behind projects and even less are they likely to make a major adjustment in their research to take account of the projects stated aims. And in many, though not all, countries the availability of funds for the support of research is likely to be tied much more closely to the excellence of the investigator or the relevance of the proposed work to national needs than it is to the context of an international programme Many of the remarkable discoveries in the earth sciences of the past twenty years have been made with little or no direct assistance from the prevailing project of the time. On the other hand, international projects can concentrate on two aspects of science which national systems are often not competent to assist—indeed sometimes seem positively to hinder: the bringing together of people from a wide variety of national and multi-disciplinary backgrounds, and the removal of blockages in making data easily accessible regardless of its national origin. On this basis alone the modest costs of a small secretariat, travel funds and data centres seem amply justified. Since all branches of science at all times profit greatly from this sort of assistance it is unfortunate that international unions have to convene working groups, write long reports and propose grandiose projects simply to maintain the rudiments of international and interdisciplinary scientific exchange.

Be that as it may, with the Geodynamics Project running out in ten months' time there is a pressing need for a new programme to be announced. Four years ago it seemed possible that the International Union of Geodesy and Geophysics (IUGG) might opt completely out of a further inter-union programme (the totally illogical way in which the geologists and geophysicists have separate unions and entirely different organisational machinery within them surely represents one interdisciplinary blockage which ICSU could do well to eliminate). In the past year and a half, however, IUGG has buried the hatchet and working groups convened by Dr H Illies of Hamburg (IUGS) and Dr C. Kisslinger (IUGG) have now brought forward a joint proposal for the next ten years. But even if the respective unions in their different ways accept the present document, it will not be until the summer of 1980 that final approval can be obtained from ICSU's general assembly, so there is bound to be a hiatus between one project and the next.

It is possible to identify three distinct threads of argument in discussions of this document. The first recognises that scientific advance is to a degree independent of international projects so simply reckons that the next ten years will build on what has already been learnt, though will doubtless contain some surprises—will in effect be a continuation of the International Geodynamics Project. The second believes that to be politically acceptable it will not be possible simply to say 'more of the same'—a new title will be necessary and new foci of attention will be called for. The Illies-Kissinger document carefully pulls together these two threads; the central theme of the proposed new programme would be 'the current state, dynamics, origin and evolution of the lithosphere, with special attention to the continental lithosphere'. (The lithosphere is the strong outermost shell of the Earth, of depth up to 150 kilometres beneath continents.) The programme proposes a change of focus and yet is a logical consequence of what has gone before.

The third line of thinking, however, says that earth scientists must respond to changing political climates, most notably in two areas: first, global resources, hazards and the maintenance of the environment; second, the need of the developing world for strengthened scientific and technological bases.

This geopolitical point of view has been put forward most forcefully by Professor L. Nicolaysen of the University of the Witwatersrand, Johannesburg. He recognises that the Illies-Kisslinger document does mention these matters, but would have them occupy a much more prominent position in project plans.

There are some fairly good administrative and bureaucratic reasons why this proposal can be resisted. It raises all sorts of questions that will offend countries' sensibilities, bring in new agencies, straddle difficult industry/academic frontiers, call for larger expenditure and so on Quite so. But it is time earth scientists faced up to the uneasy barriers that exist between pure and applied research, and it is time they recognised the unique role they can play in the developing world. Maybe they should look, for a start, at the success of the International Centre for Theoretical Physics at Trieste in nurturing developing world scientists, and should wonder whether the earth scientists might evolve a similar project.

It would be a mistake simply to avoid these issues as being too political or too complex. The unions, whilst accepting the present document as a sound basis on which to proceed scientifically, should grasp the opportunity they have to bring together a highly fragmented range of disciplines, to take greater steps to bring basic and applied science together and to start serious thinking about responsibilities in the developing world. □

US boosts military support of campus research

Defense Department support for basic science is returning to pre-Mansfield Amendment days. **David Dickson** reports

AFTER a gap of seven years, the US Department of Defense has re-established an office to co-ordinate its efforts in basic research and to stimulate contacts with university research groups.

This move, which was recommended in a report on the department's basic research released by the Office of Science and Technology Policy in Washington last week, reflects an effort backed by President Carter to restore military support for university research to the levels of the late 1960s. Two factors have brought this about: renewed pressure for technological innovation—with requirements ranging from laser weapons to high-speed integrated circuits—within the Department of Defense; and the continued squeeze on other sources of support for university research.

The clearest evidence of the new trend is provided in the budget request submitted by the President to Congress last month This asked for $436 million for basic research obligations in the Department of Defense in the fiscal year 1980, almost 10% of the total basic research budget If the request is accepted by Congress, this would mean an average increase for Defense Department basic research in the two Carter budgets (covering the period 1978–80) of 18.3%, considerably higher than for any other agency, and compared to an average increase of 11.4% for the National Institutes of Health, and 10.5% for the National Science Foundation

Military support for basic research reached its peak in the late 1960s, when the efforts of university scientists in fields such as nuclear weapons were supplemented by those recruited to support US efforts in Vietnam. Reaction against the conduct of the war, however, and the use of science in areas such as chemical weapons or counter-insurgency techniques, led to a wave of protests about military involvement on university campuses.

The protests became focused in an amendment made by Senator Mansfield to the Defense Department appropriations in 1970 Seen by some as merely a "good housekeeping" move, but by others as reflecting broader concerns about military involvement with science, the amendment stated that funds would only be provided to research projects bearing a "direct and apparent relationship" to a specific military function or operation

Although revised a year later to require merely a "potential relationship" —a criterion which is still in effect—the Mansfield amendment contributed to a rapid shrinkage in Defense Department support for basic science, which in real terms was almost halved between 1967 and 1975.

In its report, the OSTP panel, which was chaired by Dr J K Galt of Sandia Laboratories and included representatives of universities, industry and other government agencies, states its belief that the current level of funding has reduced the utilisation of basic research in the Department of Defense "below a minimally acceptable level". It "welcomes and applauds" recent attempts to reverse the previous decline in research funding

The panel says that such research is important to the department for three main reasons: to fill gaps in knowledge, to provide a source of new concepts, and to keep up with technological developments in other parts of the world "Growth for 10 years at a rate similar to the rate of decline of the past 10 years is indicated", it says The OSTP panel places a special emphasis on the potential contribution of university scientists in single-mindedly pursuing long-term goals rather than performing highly-specified research. The universities are a "logical" place to expec original and startling new ideas, it says

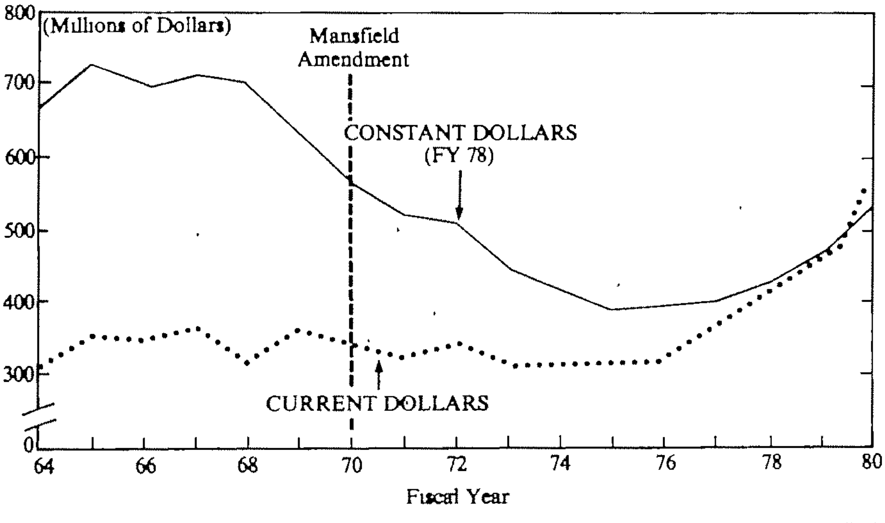
This emphasis on supporting university research is already reflected in the department's budget. Between 1976 and 1978, for example, while Defense Department support for in-house basic research was kept constant (in curren dollars), support for basic research in universities increased by almost 60%. In 1976, universities carried out 33% of the department's basic research; by 1978 the proportion had increased to 40%.

There have also been shifts in the areas of research receiving support From the US Air Force, for example there has been a decline in support for nuclear physics or atmospheric research, and an increase in materials science and engineering Mathematics however, and particularly the computer sciences continue to receive good support.

So far there has been little opposition from the academic community to the re-emerging importance of Defense Department research funds. A few re-echo the concerns of the late 1960s "The trend is part of an overall growth of military spending and militarism in this country that I consider very unhealthy for science in its broader context—it's like saying that wars are good for surgery", says Dr Charles Schwarz professor of physics at the University of California in Berkeley

And a group of scientists in Boston have recently recommended that basic research be removed from the Department of Defense budget, where they say it has a "high probability of military applicability" They have suggested that the majority of the funds be transferred to a National Technology Foundation, which would stimulate R&D in areas of technology where private industry is not yet willing to undertake the work

But most scientists are taking a more pragmatic line They point out that unlike the more applied research sponsored by the Defense Department in university-associated laboratories—suc as MIT's Lincoln Laboratory—th basic research it supports is unclassified And it also has spin-offs in other areas □



Basic research spending by the Department of Defense, 1964–1980 (Source DoD/OSTP)

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DNA risks: fears calmed, but doubts remain

EXPERIMENTS carried out by research workers at the US National Institutes of Health have provided little evidence to support fears that bacteria carrying recombinant plasmids or phages containing a eukaryotic viral genome could themselves become producers of virus particles.

However, the research workers have shown that free viral DNA is capable of producing infection in mice when injected under the skin, and that infection can also be caused by recombinant phages in which the viral DNA occurs twice in succession. They have also raised the possibility, now receiving further study, that the recombinant molecules could present a hazard by transforming cells into a tumorgenic state.

These results have emerged from a series of experiments carried out at the top-level containment facilities of Fort Detrick in Maryland as part of a programme to assess the potential risks posed by the use of recombinant DNA techniques.

A team of research workers from the National Institute of Allergy and Infectious Diseases at NIH, headed by Dr Wallace P. Rowe and Dr Malcolm A. Martin, conducted two series of experiments. One involved the molecular cloning of polyoma virus in a disabled *E. coli* plasmid vector system, and the other involved studying recombinants formed between a lambda phage vector and polyoma DNA.

In the first series of experiments, the research workers found that although all the recombinants studies contained the complete, potentially infectious viral DNA, in no case did either the recombinant plasmid, or live *E. coli* containing the plasmid, cause polyoma infection in mice.

In the second series, two types of recombinant phage were found to have formed, the most common containing a single copy of the polyoma DNA, but a second type containing two copies of the DNA linked head-to-tail in tandem. The phages containing the single copies was not infectious in mice, either when given directly, or when used to infect concentrated suspensions of *E. coli* from which the DNA was extracted and administered to the mice.

However, the phages containing the tandem inserts — theoretically more likely to cause infection since polyoma genomes could be generated by intramolecular recombination—did indeed prove to be infectious in two of five mice inoculated with the phage DNA, and in two of five injected with DNA prepared from *E. coli* infected with the phage.

According to the research workers, whose findings are about to be published in *Science*, the latter result indicates that the possible cloning of head-to-tail inserts should be taken into consideration in carrying out risk assessment analysis of the cloning of DNA, although they say that such an occurrence is highly unlikely to occur, particularly in shot-gun experiments.

The research workers say that similar results on the low infectivity of cloned genomes can be predicted for other members of the *Papovaviridae* family of viruses—including the SV40 virus—as well as most other DNA viruses, containing larger and more complex genes.

However, they draw attention to a potential hazard that might be caused by the ability of bacteria containing virus DNA recombinants to induce tumours. This hypothesis is based on the ability of polyoma DNA to induce tumours in young hamsters by transforming cells; tests of any potential hazard are now being investigated. ☐

David Dickson

Science gets new champion on Capitol Hill

IT IS unusual to find a US Congressman demanding a strong, centralised state planning mechanism, a device usually associated with the nation's ideological rivals. Just such a mechanism, however, is being proposed by a member of the House of Representatives whose voice on science policy matters will be frequently heard over the next two years: Representative George Brown of California.

First elected to Congress in 1953, Mr Brown has been chosen to chair the science, research and technology subcommittee of the House science and technology committee. The subcommittee's responsibilities will be expanded by incorporating the domestic and international scientific planning committee; and the result will be to make it broadly responsible for overseeing the nation's research efforts and science policy.

Mr Brown is unashamedly partisan. "Some people talk of science as the handmaiden of society; I want to see science as a leader in our national culture", he said in an interview with *Nature* last week.

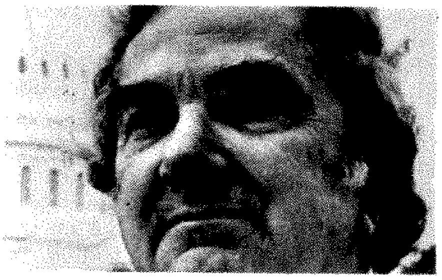
He is critical of a "laissez faire" attitude to research and development, where national concerns merely adjust the broad parameters of programmes rather than their more precise dynamics. The US, he says, lacks "a coordinated national approach to giving both direction and resources to the scientific community".

With the general pressure on research budgets, funding innovations must concentrate on the reorganisation, rather than the expansion, of resources, he says.

If this sounds a bit vague, then Mr Brown can point to his recent experience in helping steer through Congress legislation to set up co-ordinated programmes of climate and earthquake research—efforts which have earned him widespread respect in the scientific community—as well as his involvement in agricultural and environmental research issues.

But not all agree with the primacy that he would allot to science. During last year's debate on the climate programme, for example, the OSTP successfully headed off an effort to make it lead agency for the programme, a task passed to the Department of Commerce's National Oceanographic and Atmospheric Administration.

Mr Brown remains convinced that OSTP should be at the top of the pile. Although welcoming the increased funds that the science adviser has helped secure for basic science, he claims that OSTP has not confronted to the intentions and the language of the legislation which set it up in 1976.



Congressman George Brown: "I want to see science as a leader in our culture."

"We had hoped that the science adviser's office would give some coherence and strategic planning to science, and it has not done so, leaving quite a lot of unfulfilled expectations", he says.

At OSTP they see things slightly differently. "Some of these expectations were perhaps partly misplaced. We are not a kind of national clearing house on R & D", one official said last week.

Nevertheless mechanisms by which the nation's scientific efforts can be given "improved leadership" are high on Mr Brown's agenda. "The period of substantial growth experienced by science a decade ago has been extremely valuable; but it is now time to do some stocktaking". **David Dicksor**

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France attacks UN research on Mediterranean

Mediterranean countries are to slow cooperative research on pollution, writes **Robert Walgate** from Geneva

France, assisted by Spain and Italy, has succeeded in cutting three major projects from the United Nations Environment Programme's research effort on pollution in the Mediterranean. Research has been halted on pollutants in the open waters of the Mediterranean (as opposed to the coasts), on transfer of pollutants from the air to the water, and on modelling the Mediterranean ecosystem.

Last week's meeting in Geneva of the 18 countries which signed the 1975 Barcelona convention on Mediterranean pollution was dominated by money problems, in particular shifting payment for the Mediterranean action plan from UNEP—where responsibility has lain until now—to the states themselves. France, as the largest of the 18 countries, was concerned that its contribution should be well spent, and so devoted considerable efforts to analysing the research plans of UNEP's Regional Seas director, the Yugoslav Stjepan Keckes.

Keckes had developed a 13-part programme of research in the Mediterranean known as 'Med Pol' I to XIII. The first seven deal with coastal waters, and were approved by the original Barcelona meeting. It is Med Pol VIII to XIII which were added later and deal largely with open waters that were challenged last week.

The total research costs involved were relatively small—around US $1 million a year, compared with an estimated cost of some $5 billion for treating presently untreated Mediterranean sewage effluent — so some matter of principle appeared to be at stake. If the countries were serious about dealing with Mediterranean pollution, why should they be bothered by the one part per thousand of the costs that came in the form of research?

The question mark hanging over the whole meeting was precisely what principle was at stake. According to the French delegation, the question was the quality of the research teams that UNEP had put together.

Keckes had, as a matter of principle, drawn together the expertise of existing government - sponsored laboratories around the Mediterranean coast until he had some 83 participating labs in 16 countries. These varied greatly in quality, but he took the view that governments would respect the conclusions of their own labs more than those of externally imposed teams of 'experts', however good.

Further, by receiving advice and financial support from UNEP, these laboratories could be brought up to an international standard. So far, UNEP has spent some $1.4 million on equipping these labs, mostly with American equipment (which an independent panel adjudged to be the best).

By Keckes's own admission, not all these laboratories have come up to scratch. In one southern Mediterranean state, for example, UNEP equipment languishes in a filthy laboratory without supplies of water or electricity, in spite of continued assurances that the necessary facilities will be supplied. On the other hand, neighbouring states are superior to some northern countries.

Keckes is undaunted by these setbacks, seeing the treatment of the Mediterranean and the improvement of developing country science in a very long perspective. But at Geneva the French delegation took a different view. They argued that the UNEP team could only cope effectively with local, coastal sources, and that the more complex issues of open-water pollution, air transport and ecological monitoring were beyond them. France already has programmes in these areas, said the delegation, and would make its information open to UNEP.

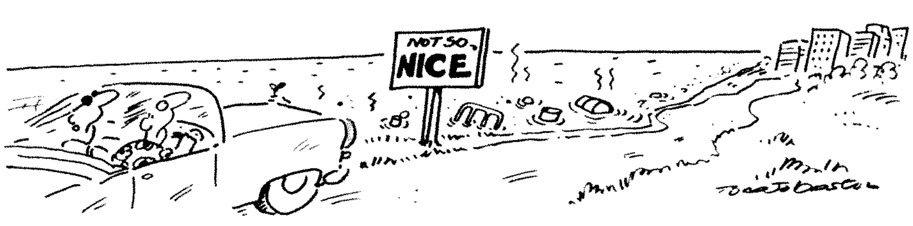
Keckes, however, thinks that the principle at stake may have been different—and closer to a question of the ownership of sensitive data. In the past he has found French officials "unforthcoming" about pollution of the Rhône river, which is one of the three major pollution sources of the Mediterranean (the others are the rivers Po—Italy—and Ebbro—Spain), and he suspects that the same will be true of central Mediterranean data. This data is essential to an assessment of the long-term effects of pollutants, particularly cadmium and mercury and polychlorinated hydrocarbons (from pesticides).

Further, the air—whose circulation over the Mediterranean is little understood—may be one of the main sources of these pollutants. According to Keckes "the input of airborne pollutants may turn out to be the major unknown parameter needed to assess the state of pollution in the Mediterranean basin". Measurements in the Baltic have shown that between 10% and 90% of heavy metal pollution comes from the air, and in the Adriatic it has been observed that there is some 300 times more heavy metal in the top metre of the sea than in deeper layers. Polychlorinated hydrocarbons affect the reproductive cycle of fish, and so could have a long-term effect on Mediterranean fish-stocks—a matter of more interest to southern nations with a comparatively greater reliance on fisheries, than to industrialised northern states. Nonetheless, the air-sea research programme has been cut.

The French view is that not only is the Mediterranean team of poor quality to undertake such vital work, but the necessary instrumentation and methodology is not yet available to make an accurate assessment. Keckes admits that measurements so far are only accurate to an order of magnitude, but believes that it is necessary for the whole programme to proceed in parallel, so that each part can learn from the others. He hopes that the deleted research can be revived in UNEP's long-term programme for the Mediterranean, which will be agreed in 1981.

The Regional Seas team is not discouraged. On the contrary they had been led to expect far worse. Research is only part of the Mediterranean Action Plan, which includes 'priority action' (inexplicably including development of alternative energy sources) and the 'Blue plan' for coordinated environmental management and development of Mediterranean industry. The balance between these programmes has been maintained.

In the final analysis, the budget for the Mediterranean Action Plan for 1979 and 1980 was put at $6.4 million. Of this, $3.3 million will come from the Mediterranean states and $1.6 million from UNEP. Within this the total budget for Med Pol for two years will be some $880,000. The rest will be provided by other UN agencies such as the World Health Organisation and the United Nations Development Programme for parts of the programme related to their own interests. □



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Soviets soften on psychiatry?

REPORTS from Moscow suggest that, for the first time, the Soviet psychiatric establishment is willing to engage in cautious dialogue with dissidents over alleged cases of political abuse of psychiatry.

Since its foundation in January 1977, the unofficial Moscow "Working commission for investigating the use of psychiatry for political purposes" (an adjunct of the Helsinki monitoring group) has been publishing regular reports of such cases. In Bulletin 13 (November, 1978), it reported the case of Yurii Zakharovich Valov, hospitalised apparently for having made repeated protests to the media against what he saw as unfair dismissal from his job in an engineering works. The diagnosis was not revealed; however, it was known that Valov had been prescribed heavy medication—including insulin and chlorpromazine.

Dr Aleksandr Voloshanovich: asked to advise

The latest bulletin, number 14 (January 1979), takes up the case again. According to Soviet practice, Valov's case was to be reviewed by a medical commission on 7 December. As well as the regular establishment doctors, a representative of the working commission was invited to be present—Dr Aleksandr Voloshanovich, a psychiatrist, who has been acting as a consultant for the working commission since last April.

The official report on the Valov case stated that Valov was in need of "biological treatment, psychotherapy, and subsequent socio-psychiatric measures". The exact grounds for this recommendation are not entirely clear though "personality disorders" have been mentioned.

Dr Voloshanovich agreed with the need for psychotherapy and "socio-psychiatric measures" (whatever they may be), but he denied that hospitalisation and the use of neuroleptics were indicated.

This is by no means the first case where extreme psychiatric measures were employed, on political grounds, against a person suffering from a mild psychological disorder. The invitation to Dr Voloshanovich to sit in on the case may well reflect a double approach to the working commission—a desire for cautious dialogue combined with a wish to obtain at least a partial confirmation of the official verdict.

Vera Rich

Shortage of technologists in USSR

INTEGRATION of research and industry is a fundamental tenet of Soviet science policy. It is the rationale behind the establishment of a network of 'science centres' throughout the country, and the occasional Party censure of individual research bodies which neglect the industrial problems of their local area.

In recent months, however, official emphasis has been turning away from the role of the scientists in the 'scientific-technological revolution' to that of the technologists. Last July, Mr Brezhnev told the Plenum of the Central Committee that the shortage of middle-level managers and department heads in factories was disquieting, while in agriculture only 40% of such posts are filled.

Last week, *Pravda* took up the case of other organisations including scientific research institutes, where there is also a serious lack of middle-level staff. The problem seems to be a dual one: the actual training of the required personnel and their deployment once they have qualified.

Technical college admissions, says *Pravda,* are constantly increasing (almost 1.5 million new enrolments last autumn). Nevertheless, it comments, "training has to be formulated taking into account long-range forecasts of the development of science and industry", which suggests that certain courses need re-designing and/or upgrading.

At the same time, the deployment of the present work-force of middle-level technologists—the "sergeants of industry"—is far from at its best.

The *Pravda* editorial (a traditional way of launching a new efficiency drive), calls for the relevant Party and Trade Union Committees, and the All-Union and Republic Ministries of Higher Education, to "analyse and eliminate" these defects.

Vera Rich

British scientists help Chinese set up new university

THE UK is to help China set up and run an English-speaking technical university. The idea for such a collaboration was first raised last November when a high-level delegation from the Chinese Academy of Sciences visited the UK. British officials showing the delegation around scientific establishments were taken by surprise by the proposal, which was announced towards the end of the visit. It was not clear, according to John Deverill of the Royal Society, whether the Chinese decided to propose the cooperation on the spur of the moment or whether they had planned it before their visit.

The university is to be established at Chengtu, the capital city of Szechuan, a province of central China covering 6% of the country's land area and containing 10% of its population. Chengtu Technical College, which has 2,800 students, 1,190 academic staff, and five departments is to be expanded to include 5,000–6,000 undergraduates, 1,500 postgraduates and 13 departments and given university status. The vice-chancellor will probably be Mr Ma Shih-tu, vice-president of the Chengtu branch of the Chinese Academy of Sciences.

The key UK bodies involved in the operation will be the Royal Society and the British Council, though no one is yet sure what it all entails. "The Royal Society is not set up to start universities in other people's countries", Mr Deverill says, but he thinks it could be arranged for academics to spend some time—from one to three years—at Chengtu helping to set up new departments and start new courses. The British Council will probably help establish English courses and arrange for English teachers to spend time there. The administration and funding of the university however, are likely to be left to the Chinese. A delegation of British officials and academics now visiting China is assessing more precisely how the UK can help at Chengtu.

Chengtu will be unique as the only exclusively English-speaking university in China. The Chinese Academy of Sciences runs two other partially English-speaking universities at Hangchou and Hofei, and one partially Japanese-speaking university at Harbin. The Chinese Ministry of Education used to run French and German-speaking universities in Shanghai. There is a possibility that both these could be restarted with French and German help.

Judy Redfearn

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news in brief

Profit plummets after nuclear problems: Siemens, the West German transnational electrical company, has had a first quarter slowing of its sales, orders and profits because of difficulties with its subsidiary, Kraftwerk-Union, a nuclear contracting firm. Losses in KWU caused by problems in Iran, Brazil and a partial freeze on nuclear construction at home have offset an 8% increase in new orders for the rest of the Siemens group. Failure to authorise new power stations is "driving West Germany to catastrophe", said Dr Bernhard Plettner, Siemens chairman. As a way out, Siemens is trying to win a contract in Argentina to build a second nuclear plant in addition to a KWU facility in operation there since 1975. But as part of the deal, the Argentine government is demanding additional facilities to enable it to produce plutonium. The Bonn government faced severe criticism from the US for making this kind of arrangement with Brazil last year.

Swiss to vote on reactor safety: Radical new licensing measures for reactor safety to be inserted into the Swiss constitution will be voted on by the electorate on 18 February. The referendum includes: proposals for autonomous regional approval within a 30 km radius of all proposed sites; accident compensation without limits; 25-year operating concessions for both plants and the associated storage of waste; requirements that all catastrophe precautions be made public; cessation of construction or operation if at any time safety guarantees are withdrawn; and a bridging rule that will require existing plants to reapply for approval under the new rules within three years. The result of a five-year effort by a coalition of Swiss anti-nuclear groups, the campaign got boosts from a successful site occupation in 1975 at Kaiser Augsp near Basle and the emergence of a strong women's contingent opposed to nuclear power. Swiss authorities believe that if the vote goes in favour of the proposals, three plants—at Leibstadt, Kaiseraugst, and Graben, where local opposition is particularly strong—will be blocked. The bridging rule could mean that existing plants at Beznan, Muehleberg (pictured above) and the brand new 920MW plant built by the West German firm KWU at Goesgen could be closed.

Energy conservation month: October 1979 has been designated International Energy Conservation Month by the International Energy Agency (IEA). The 19 member states of the IEA are organising a variety of events to take place during October with the aim of increasing public awareness of the need for energy conservation. Several international conferences are being organised including one on the use of urban waste heat in Tokyo last October, one on energy management to be held in Birmingham, UK on 9-11 October, one on the energy conservation policies in several countries to be held in Sweden and one on the use of solar-heated water to be held in Spain.

In its national programme, the UK is laying particular emphasis on energy management, an area in which it feels it has gained some expertise in recent years. During October it will also be relaunching the "save-it" campaign for 1979-80 at a cost of £2 million and publishing the report of the Advisory Council on Energy Conservation.

Spain will promote the month by organising a 'bicycle day' and in Switzerland local authorities will be able to measure heat losses from buildings by means of aerial photographs. In general, the Mediterranean members of IEA such as Italy, Spain and Greece are hoping to demonstrate the uses and importance of solar power while some of the colder countries, for example, Denmark and Canada are laying more emphasis on conservation in buildings.

Aid to Vietnam: Vietnam's scientists, known to be desperately short of scientific equipment, are to be helped by a British charity. Medical and Scientific Aid for Vietnam has collected £8,000 to buy equipment for biologists in Vietnam. A spokesman said the money was raised in a year. Of the £8,000, £2,500 was provided by another charity, War on Want. The money has been used to buy gel electrophoresis and column chromatography equipment for work on protein separation; a spectrophotometer for routine analysis; and some general laboratory glassware. These items will help equip one of the country's newest laboratories—that of biochemistry and food chemistry in the Vietnam Scientific Research Centre in Ho Chi Minh City (formerly Saigon). The laboratory is doing agricultural research and scientists will be investigating disease resistance in rice as well as looking at cheap sources of nitrogenous fertiliser.

Bombay magnetic observatory threatened: Construction of India's largest fertiliser complex at Thal-Vaishet threatens the Colaba-Alibag magnetic observatory 5 km away. It is feared that magnetic observations taken over a continuous span of 133 years will be disrupted by electrical interference from the plant. Dr Stuart Malin of the International Geological Survey described Colaba-Alibag as "probably the most valuable magnetic observatory in Asia", but added that "the fertiliser factory is undoubtedly more important to India than the observatory". The factory has been opposed by the Save Bombay Committee, a group concerned with the pollution risk to 37,000 people 10 km downwind from the site. The committee will take the case to the Bombay High Court.

Bangladeshi centre for appropriate technology: Bangladesh is to set up a National Centre for Appropriate Technology at Bangladesh University of Engineering and Technology (BUET) that will cost $4 million and will be completed in two phases: the first in 1980 and the second in 1985.

The broad objective of the centre is to identify, develop and adapt technologies appropriate to the basic needs of the people of Bangladesh. Its studies are to be sponsored by government, autonomous bodies, private organisations and international agencies. It will provide training and research facilities and will maintain contact with similar organisations throughout the world.

Its major task will be to co-ordinate activities between scientific institutions and organisations in Bangladesh. Agriculture is the first area it will be considering. It is proposing to set up regional centres at the Agricultural University, the Academy for Rural Development, the Agricultural Institute, the Rice Research Institute and at three engineering colleges. It will monitor the diffusion of technology to the regional centres. Networks of institutions are also to be set up for industry, communications and energy with the new national centre as the focus.

The centre will also work on the design and fabrication of new products and the modification and adaptation of imported goods. Its aim is to provide government with policy alternatives on the import, adoption and adaptation of foreign technology.

Flaws in GMAG's guidelines

‘We urge that time be allowed . . . before any firm decisions about major changes in the approach to categorisation are made’ — Royal Society

• A statement issued by the Council of the Royal Society and prepared by an ad hoc group chaired by Professor W. F. Bodmer. Other members were: Sir David Evans, Professor D. C. Phillips, Dr M. G. P. Stoker, Sir Frederick Warner, and Sir Robert Williams.

WE welcome the attempt of the Genetic Manipulation Advisory Group's paper (*Nature*, 9 November, page 104) at a more rational approach to the categorisation of the experiments which come under its jurisdiction, using the framework of risk assessment. The chemical and nuclear industries have considerable experience of quantitative risk assessment, based on fault-tree analysis, and hazard and operability studies, backed by extensive data on reliability. In the longer term we believe that advantage should be taken of this experience and so we are requesting the Royal Society's recently established Risk Study Group on the Assessment and Perception of Risk, under the chairmanship of Sir Frederick Warner, to include the GMAG paper and the application of risk assessment techniques to genetic manipulation in its considerations.

Even in areas such as the chemical and nuclear industries, where extensive background data are available, there is often disagreement among experts. We believe that the present knowledge of the quantities needed for a quantitative risk assessment in the area of genetic manipulation is quite inadequate for this now to form a basis for categorisation of experiments. The absolute risk associated with any experiment involves the product of many probabilities (all, of course, less than one) some of which are known and known to be low, and so this risk may in many cases be estimated to be very low, even given our present incomplete knowledge. Categorisation of experiments, however, requires the estimation of relative risks, or ratios between probabilities which in most cases will be small, and, at the same time, quite imprecisely defined, and so we do not see how these ratios can yet be calculated with sufficient accuracy to be useful for categorisation.

Particular areas of ignorance are the probabilities of human error and the real risks associated with the ingestion of the experimental product, namely the host carrying a recombinant vector. In the case, for example, of vaccine development such as for polio, which probably involved much greater potential risks than most work with recombinant DNA, extensive data were collected on the *in vivo* effects of potential vaccines in animals and humans, on the basis of which the magnitude of the risks could be confidently assessed.

There is an urgent need for more comparable data on the recombinant DNA products and on their associated hosts and vectors, such as *E. coli* K12 and its mutants, phage lambda and other plasmid vectors. Until such data, and other relevant information required for quantitative risk assessment, are available we believe the approach suggested in the GMAG paper, and appropriate developments of it, should be used for studies of the existing categorisation, rather than for categorisation itself. If, for example, further testing fully supported the present evidence that *E. coli* K12 could not be converted into an epidemic pathogen by recombinant DNA techniques, then it might be possible to assign nearly all recombinant DNA experiments using normal *E. coli* K12 host-vector systems to category I, or at most category II.

The real risks, if any, associated with most experimental products of genetic manipulation are, as already pointed out, the most difficult to assess since they are all, so far, conjectural. While it is appropriate to encourage research in areas which might be thought relevant to the assessment of these conjectured risks, as in the GMAG paper, we believe that the discussion of such conjectured risks should be adequately documented by reference to the scientific literature and subject to comparable standards of scientific scrutiny.

The discussion in the GMAG paper falls far short of these standards. For example in describing risks (the section headed "The Conjectured Hazards") there is a progression from "conjectured" through "likely" and "potentially severe" to "possible" with no justification whatsoever. The mere existence of a list of conjectured risks introduces the notion of their probability, when some may even be impossible. In other contexts, risks of this sort have been classified, by consensus of those knowledgeable in the field, into (1) negligible, (2) possible but perhaps doubtful, and (3) definite though possibly small risks. It might be instructive to do the same with some of the conjectured risks that have been attributed to work with recombinant DNA.

The difficulty in applying the suggested new approach to categorisation is illustrated by some of the chosen examples in the GMAG paper. "Self-cloning", for example of *E. coli* K12 genes into *E. coli* K12 host-vector systems, is assigned to category I and made the subject of extensive notification and detailed reporting to GMAG, whereas the Health and Safety Executive's definition of genetic manipulation, accepted as unchanged by GMAG, specifically excludes such experiments from its remit (through the use of the phrase ". . . incorporation into a host organism *in which they do not naturally occur* . . ."—our italics).

We deplore any extension of GMAG's remit, implicit or explicit, especially into areas where there is evidence that the risks are negligible and which form a significant part of experimental work in modern biology. Any such extension should be subject to very careful further consideration and justification and should not arise automatically from the adoption of a new procedure.

It is also not at all clear why experiments involving the expression of cholera toxin genes in *E. coli* K12 need to be performed under conditions which are considerably more stringent than those required for work with *Vibrio cholera itself*. We urge that any recommendation for a higher level of containment for recombinant DNA work with a pathogen, than for work with the pathogen itself, at equivalent doses, be carefully justified. In many cases involving, for example, work with certain animal viruses it is likely, as has often been pointed out before, that recombinant DNA experiments with parts of the viral genome will entail much less risk than work with the whole virus.

We believe it to be very important that GMAG consider its procedures carefully in relation to the new US National Institutes of Health (NIH) Guidelines which came into effect on 2 January 1979. These new guidelines were prepared after extensive open, documented and subsequently published consultation with many groups in the United States and abroad, including in particular Europe and the United Kingdom, especially through the European Molecular Biology Organisation. In the earlier considerations of the

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problems posed by genetic manipulation, following the "Ashby" (Cmnd 5880) and "Williams" (Cmnd 6600) Reports, the approach followed independently by the United Kingdom was quite close to that followed in the United States and indeed at times in the forefront.

The new NIH Guidelines, which are being adopted in some form by most other countries that are advanced in this field in Europe and elsewhere differ, however, quite significantly not only from the new GMAG proposals, but also from some of its current practice. They differ, for example, with respect to self-cloning experiments and in the use, in general, of set categories for given types of experiments, rather than accumulated case law. A set list of categorisations obviates the need, once an institution is appropriately registered, for repeated referral back to a central authority before starting any particular experiment. Now that a significant amount of case law has accumulated, we urge GMAG to consider following this approach for an increasing proportion of experiments under its remit, and so simplify its activities and those of the people it serves, while in no way jeopardising recommended standards of safety.

We believe that in cases where GMAG's procedures lead to substantial differences in categorisation from those recommended by the NIH Guidelines, the difference must be carefully justified. A lack of comparability between procedures in the United Kingdom and elsewhere, especially in the United States, could seriously jeopardise this country's research and development efforts in this important area of work and in particular, threatens our contribution to extensive international cooperation in research in this field.

In this connection we would draw attention to the coordinating activities of COGENE, the Committee on genetic experimentation set up by the International Council of Scientific Unions. It should be noted that COGENE jointly with the Royal Society is organising an important meeting in April 1979 to bring together those concerned from many countries to consider these problems.

We have emphasised the need to obtain much more quantitative data to form the basis for a quantitative risk assessment. The pooling of data on risks with the US and other countries, as is done, for example, in the nuclear industry, would greatly extend the available data base needed for a proper quantitative risk assessment.

The timing of requests for responses to the GMAG paper, just before Christmas, and its earlier working paper in the summer, has been unfortunate and the period allowed for a response very short. It is not clear why such a major change in approach should be treated with such urgency. We urge that time be allowed to collect more risk data, to assess the risk assessment approach in relation to current categorisation, and to assess the impact of the new NIH Guidelines, before any firm decisions about major changes in the approach to categorisation are made. □

Arithmetical perils

Thomas H. Jukes

RECENTLY I gave my class in elementary haematology a midterm examination that included a 'free gift' question: "A patient has an erythrocyte count of 4.57 million per cubic millimetre and a blood haemoglobin level of 14.1 gm per decilitre. What is the mean corpuscular haemoglobin content?" The answer, in picograms per erythrocyte, routinely appears on computerised read-outs of blood analyses. Most of the class refused to attempt the question. I think they felt its intricacies were so mentally disturbing that to tackle it would interfere with their answering the rest of the examination.

Arithmetical problems also arise with residues in food. The Delaney Clause of the Food Additives Amendment is interpreted as prohibiting the presence in food of any added carcinogen at any level, no matter how low. Supporters of the clause often say that there is no "noncarcinogenic" level of any carcinogen. This "no-threshold" concept is sometimes arbitrarily extended to toxicological effects other than cancer. This controverts the essential basis of the science of toxicology that the dose determines the poison. As a result, per cent, parts per million and parts per billion (ppb) merge hazily into each other in the minds of many people. I wonder how many mathematicians would willingly drink a glass of water if they were told it contained 30 ppb of arsenic? Yet the human body normally contains 40 to 300 ppb.

There was a protest about the arsenic content of California wines in one of our local magazines, with the usual charges about the malfeasance of those who would poison for profit. The authors vividly described the agonies resulting from arsenic overdosage, without mentioning the quantities involved. They also hinted darkly at a University of California cover-up of the analytical results on wine. I pointed out that the offending wines had a range of 5 to 116 ppb of arsenic and that marine fish contain 2,000 to 8,000 ppb. It was no use; the authors stuck by their smoking guns, and talked about arsenic and cancer, and the rights of consumers to be informed of "the complete known and suspected contents of all food, beverages, drugs, water and air." I hope that they stop by the health food store for some dried kelp, and ask for information on its arsenic content.

The arithmetic of selenium has stumped the finest minds in the FDA for about 20 years. It seems that they think selenium is a carcinogen. It came into initial prominence in the 1930s as a teratogen in farm animals that were fed wheat from seleniferous soils in the Dakotas. In 1957, it was found at the National Institutes of Health, and simultaneously by our group at Lederle Laboratories, that selenium was an essential nutrient, and within a few months, selenium deficiency in farm animals was reported from many parts of the world, especially in New Zealand, but also in the USA, despite the excess in the Dakotas. Mindful of the Delaney Clause, the FDA refused to permit addition of selenium, as salts, to animal feeds. Of course, they could not stop the use of fishmeal as a source of selenium. A little arithmetic would have helped: about 0.1 to 0.3 part per million of selenium in the diet is essential, Delaney or no Delaney, and 5 to 10 ppm is toxic.

The emotions aroused by arithmetical amblyopia reach a peak in disputes over fluorine. A Pittsburgh judge has just acceded to impassioned pleas that fluoridation of the local water supply be halted. The water contained 0.4 ppm of fluoride before fluoridation, and the procedure that was stopped raised this to 1.0 ppm. Apparently the added 0.6 ppm was ruled to be deadly, for some nonarithmetical reason. Incidentally, seawater contains 1.4 ppm and supports a flourishing fauna, including creatures which have splendid teeth.

correspondence

Researchers and the SRC

SIR,—We are a group of non-tenured research faculty who have been working for the University of Sussex for periods up to and exceeding ten years, during which time the university has benefited both from our skills and reputation and from the capital funds attracted by our research.

We have read with interest the recent articles in *Nature* which discussed the problems of people in our situation and would like readers to be aware of our existence as a group and of our position on the issues. We feel that the problem—while certainly a national problem—can, in fact, be solved by local action at university level: the issue is not one concerning redundancy payments, but that the university can, and should, provide permanent tenured employment for those of us who have demonstrated our proficiency and usefulness in the past.

The discussion in *Nature* has so far been concerned with researchers who derived their support from the MRC but there are many who have been supported for long periods by the Science Research Council. The SRC seems to support fundamental research in this country on the hypocritical assumption that it is the universities and their tenured staff who are carrying out the research. In fact, because of the increasing complexity of research projects and the increasing demands on the time of tenured faculty, it seems that no university group can perform any really ground-breaking research without the help of some full-time, post-doctoral research personnel. Under present conditions these research personnel are almost always non-tenured. In spite of the essential role played by these non-tenured researchers in doing the actual research the SRC maintains a policy of not supporting non-tenured research workers beyond a certain age.

In the past there was enough turnover in university and national laboratory personnel so that most non-tenured research workers eventually found a post. Also, the University Grants Committee used to provide funds to allow research projects to be "taken over" by universities but this no longer occurs. Many SRC officials, and perhaps the Council itself, still seem to feel that the universities are under an obligation to give permanent employment to research workers even though the universities claim this is impossible to do.

Even when they are in a position to make appointments the universities are not living up to their responsibilities. The majority of recent new university posts have been limited to the first few points on the lecturer's scale, thereby restricting them to people about thirty or younger. One of us was told by a professor who had just made an appointment, that he did not think any university would ever offer him a lectureship because of his seniority—referring both to his age and abilities. On a less personal level, the university here has refused to give any undertaking, in negotiations with the AUT, to show some preference to older people in temporary positions when and if new posts become available.

It is clear that in many cases the SRC is aware of the current situation. One of our members is currently supported under a research grant which contains a special condition that requires the university to employ only that particular individual as the researcher on that project, thus explicitly recognising his essential role. The SRC must however face the fact that at the moment scientific research in this country is conducted on an unconscionable basis, with untenured research personnel being treated like serfs in a feudal society, rather than the workers providing the intellectual capital which is essential to the future of a society which prides itself on its democratic traditions. The present organisation of research is basically inefficient and unstable and cannot be continued much longer: the conditions of employment of non-tenured research workers would not be tolerated in any other sector of our society

Present SRC policies include: age restrictions on appointments to SRC establishments; age restrictions on the non-tenured research personnel supported by SRC grants; attempts to force the universities to "take over" research contracts and personnel while the UGC refuses to provide the resources for such "take overs"; and restriction of the right to apply for SRC research grants to tenured members of university faculties. These are making an already difficult situation incalculably more damaging than it has to be.

There is evidently a need for basic research if Britain is to maintain any sort of competitive position in the developed world. The only way we can compete in international markets is by making maximum use of the skills and creative abilities of our people not by discarding those of us who have mastered and perfected some of the most sophisticated skills necessary for complex modern technologies.

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Seismic prediction at Pozzuoli

SIR,—Tazieff (8 September 1977, page 96) makes accusations that I deliberately put about wrong data in 1970 concerning warning signs that an erruption could occur in the vicinity of the city of Pozzuoli. This is not the first time he has made this statement and I have already answered him in a technical note presented to the Pontanian Academy in 1973, a copy of which I sent to him. I can state that it is completely untrue that I was the source of bulletins or news of an impending eruption in Campi Flegrei. Indeed to the best of my knowledge no such news was spread at all.

Perhaps I may go further. Tazieff speaks of false claims of a "conspicuous temperature rise of the Solfatara's fumaroles"; but in 1970 I wrote "geothermal measurements at the Solfatara have not shown up to June 9 (the date I corrected proofs), any sensible variation in temperature". Tazieff further speaks of false claims of extremely shallow earthquakes, claiming his team disproved their existence. A paper by I. Guerra *et al.* in *Quaderni della Ricerca Scientifica* No. 82 (1972) reproduces the seismograms from these quakes and mentions a "superficiality of foci". Finally Tazieff says that the vertical ground movement or brady-seism was no faster than usually noticed over the centuries as opposed to claims that the ground had been pushed up one metre in a "very short while". Readers can judge for themselves by looking at Casertano *et al.* (*Nature* **264**, 161–164; 1976) whether the motion was average —in my reckoning it went up to 60-70 times average.

During my involvement in these matters I acted according to my moral duties without any external pressure. Only after the order to move out of tottering houses (not the city, as Tazieff declares) could I rest peacefully, and even then of course hardly with much pleasure at seeing poor people evicted. In fact eviction saved lives, maybe as many as fifty because buildings fell down in 1970 and 1972. Tazieff indeed supported a motion on 18 March 1970 which excluded the possibility of people reinhabiting old crumbling houses.

Very briefly, my involvement was this. I first reported the inversion of the Phlegrean brady-seism to the authorities on 12 February 1970, mentioning a ground upheaval of about 80 cm in little more than a year. I warned of the possibility of seismic phenomena and that even the continuation of the brady-seism might unsettle the buildings in the area At the first shock (1 March 1970) a decree evacuating tottering buildings was issued, as a precaution. 650 shocks were recorded up to 16 November 1970, six felt in Pozzuoli itself at up to IV on the Mercalli scale. Three died in November from falling masonry and rocks. In my letter of 12 February I had foreseen a seismic, not an eruptive phenomenon.

For the sake of brevity I have only refuted a part of Tazieff's gratuitous statements: it would be possible, quoting published papers to amass much more scientific criticism. Tazieff states arbitrary conclusions in order to give value to the serious charge of a supposed participation in a "construction operation". He gives no direct proof, nor does any exist, while the indirect ones he reports are wrong or deliberately altered. Therefore all of his statements are nothing but products of fancy.

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news and views

Chromosomal aberrations at very low radiation dose rates

from John R. K. Savage

ONE effective way of determining whether an individual has been exposed to ionising radiation is to look for an increase in structural abnormalities in the chromosomes of their white blood cells after the cells have been induced to divide in the laboratory. The exposure to radiation produces chromosome damage which shows up at mitosis as structurally abnormal chromosomes in the resulting daughter cells. In certain cases it is even possible to calculate the dose of radiation received (Dolphin, Lloyd & Purrott *NRPB Report* R13; 1973; Dolphin, Lloyd & Purrott *Health Phys.* **25**, 7; 1973). (Chromosome-type aberrations following radiation fall into two main categories. Asymmetrical, which always result in an acentric fragment and are readily scorable with conventional staining. These are also termed 'unstable', as few cells carrying them can survive cell division. Symmetrical aberrations, on the other hand, do not result in acentric fragments and relatively few are scorable without recourse to special staining methods. Such aberrations are termed balanced because they do not pose any mechanical problem or lead to genetic loss at cell division and so the changed chromosomes can be passed on to the cell progeny (Savage *J. med. Genet.* **13**, 103; 1976)) This method has chiefly been used to detect acute radiation exposure, usually at levels above the permitted maximum, although the data are often difficult to interpret in cases of accident or environmental exposure where the ideal methodological conditions are impossible to obtain. In this issue of *Nature*, however (page 531), H. J. Evans and colleagues have shown that the technique can be successfully used to demonstrate a cumulative effect of very low doses of radiation over 10 years in nuclear-dockyard workers who have been exposed to doses well below the internationally agreed maximum permissible level of 5 rem per year.

At very low doses, or with very long exposure times, when a long period is required to accumulate a significant dose, many of the radiobiological problems (such as dose rate or dose fractionation) associated with the effects of large acute doses on aberration yield disappear Uniform exposure throughout the body also becomes less important as circulation, and the mixing of the lymphocyte pool, will tend towards greater dose uniformity in the sampled population Similarly, differences in the mode of formation of aberrations by radiation of different qualities (which shows up as the degree of curvature in the dose–response curve) are reduced, and all categories of aberration increase linearly with dose.

Cumulative doses over long periods have problems of their own however. For example, after an acute dose, there is a gradual reduction in asymmetrical (unstable) aberrations with time *in vivo*, as a result of the loss of aged cells, accompanied by an influx of 'normal' cells by division of lymphocyte precursors These 'normal' cells will of course also include the progeny of those cells with symmetrical aberrations The half-life of unstable cells in circulating blood is around 3 years (Dolphin *et al. op. cit.*), so with prolonged exposure there is a continuous loss of damaged cells at the same time as new damage is being generated

The effects of long-term exposure are complicated by the fact that the exposed subject is ageing. If there is any change in the spontaneous frequency of aberrations with increasing age (and there is evidence for this in man), or if there is any change in intrinsic radio sensitivity (very little within the age range considered here (Evans in *Chromosomes and Cancer* (ed. German) 191, Wiley, New York, 1974), the observed aberration yield may well show an increase with accumulated dose since larger doses will be received by older subjects, provided they continue at risk.

Such complications must therefore be eliminated before a positive dose effect can be established This has been done by careful statistical analysis in Evans *et al.*'s paper, and a significant effect for unstable aberrations results Aneuploidy, and chromatid-type aberrations, neither of which are likely to be the result of radiation exposure (in this experiment at least) did not show any such dependence upon dose, although both showed positive increase with age

The absence of a significant effect for symmetrical (stable) aberrations is puzzling, and cannot be entirely explained away by the difficulties of detection, since one assumes that scoring efficiency for this class is constant at each sampling time Furthermore, such aberrations are not expected to be selected against by the same mechanisms which eliminate unstable forms, so one might have anticipated accumulation at a faster rate. The application of modern banding techniques now enables these types to be scored with high efficiency, so such questions as relative selection could be solved Certainly such forms persist for up to 60 years in dermal fibroblasts (a very slow turnover cell population) following therapeutic radiation (Savage & Bigger in *Mutagen-induced Chromosome Damage in Man*, 155, University Press, Edinburgh, 1978), though some recent experiments in Syrian hamster skin (D. Couzin, unpublished) suggest that there may be a gradual elimination even of symmetrical forms with time *in vivo*.

The observed rate of aberration accumulation (which equals the dose–response curve in this case) is probably some function of the turnover rate of the cell population Experiments with chronic γ-irradiation in plants (Evans & Sparrow *op. cit.*; Sax *Science* **112**, 332; 1950; Sparrow & Evans *Brookhaven Symp. Biol.* **14**, 76; 1961) have shown that aberrations can reach an equilibrium frequency, the level of which is influenced by both dose rate and cell kinetics Possibly it is the very slow turnover of lymphocytes that allows an accumulation to be recorded

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In which case the picture might be quite different for other tissues in these workers, particularly in those with a fast cell turnover This must be borne in mind when extrapolation is made.

It is important to note that the corrected results represent a finite 'population' trend over several years. Similar findings within an individual, sampled on successive occasions, would be almost impossible. The magnitude of a Poisson confidence interval at this sort of aberration frequency is not generally appreciated. Even the fourfold increase above background found here would not be detected without a phenomenal amount of scoring, as the authors point out.

The principal significance of the findings lies in the demonstration that effects at these very low dose rates can be found if care is exercised in the analysis to eliminate confounding factors.

The increase reported here has been established for visible unstable types which are invariably cell lethal. We may infer that similar increases probably occur in stable types and in some changes too small to be detected by conventional methods. It is these transmissible types where any genetic significance must be sought. However, their importance depends not only on the nature of the changes but where in the genome they occur and also in what cells they are found. For example, very gross chromosomal changes, orders of magnitude above those found here persist in the fibroblasts of human skin for many years following therapy without impairment of other functions or any problems for the individual. As pointed out above the rate of accumulation is likely to vary between cell systems and one will want to know what happens in other tissues, such as testis, before a real evaluation of any possible hazard can be made. ☐

Sulphide ore deposits

from J. P. N. Bedham

IT is rare that the exponents of so observational a science as geology can have the satisfaction of saying 'we told you so'; rarer still does the prediction turn out to be lucrative. The discovery of 'massive' sulphide deposits on the East Pacific sea-floor by the CYAMEX team fulfills just such a prediction. To be sure we have had the stupendous reserves of the Red Sea brines for some time, but, despite the denials of Cronan and others, there always remained the lingering doubt that in so small an ocean, so close to land and so near to major salt deposits something odd may have happened. What was needed to dispel this doubt and to satisfy the financiers of deep sea research programmes that they may be on to a winner has now been found

What were the predictions based on? It has been accepted for some time now that ophiolite complexes are the only parts of oceanic crust to have escaped destruction in subduction zones. Many of these complexes contain copper-zinc-iron sulphide ores. Indeed one—the Troodos in Cyprus—has been mined for so long that there is debate as to whether the metal gave its name to the island or *vice versa*. The Troodos ores occur in lenses (1 to 10 million tons) capped by ochres (ferruginous muds), cherts and umbers (manganiferous muds) They have long been recognised as chemical sediments. The massive sulphide lenses are underlain by disseminated mineralisation in highly altered host-rocks. These are always pillow lavas and are usually the earliest in the sequence—that is, they were formed at the axis of a spreading-centre. Their alteration was caused by a hydrous fluid at temperatures ranging up to 500 °C. Less intense hydrothermal alteration has been recognised throughout the upper 3 km of the ophiolite. Such are the field observations supporting an origin of the ores at the spreading-centre by precipitation from an exhaled fluid. However, the problems of sources of the fluid, metals and sulphur and of the mode of precipitation required an experimental approach.

Lister has shown that sea-water can interact with lava to generate an ideal potential ore-fluid. The interaction cools and hydrolyses the lava and the fluid becomes hotter, more concentrated and acidic. Such a fluid can selectively leach base-metals from rocks and transport them in solution as chloride complexes. The rate of cooling of newly generated oceanic crust requires that the predominant heat loss is by convective circulation of sea-water. Spooner and others have shown convincingly from isotopic studies that the water which altered ophiolite piles was essentially sea-water. Both Lister and Spooner, by separate approaches, have argued that the data require many rock-volumes of water to have traversed the altered strata. Models of hydrothermal systems have shown how water brought into rock by downward percolation over a wide cachement, is exhaled at the thermal centre. Corless has shown that the abundance of many elements in the oceans may in fact be controlled by hydrothermal processes and not by continental run-off as has been previously thought. These experimental data have allowed a whole new perception of the interdependence of hydrosphere and ocean crust.

Sato derived a series of elegant models to show how hot, metalliferous, acidic brines could be either more or less dense than sea-water, this depending on the balance between temperature and the amount of dissolved solids. Interaction of such brines with the effectively infinite reservoir of sea-water would result in rapid cooling and dilution. Such conditions would destabilise the chloride complexes causing the metals to precipitate. The lower density fluids would disperse, precipitating their metals over a wide zone. Such a process explains well the pronounced metal enrichments in sediments around all the mid-oceanic ridges. It could indeed account for the metal content of manganese nodules. The denser fluids would remain on the sea-floor and flow down into the nearest topographic low, there to precipitate their metals. In many cases the site of exhalation may itself be a depression and the metals would accumulate above their feeder.

Three sources for the sulphur have been proposed—the fluid itself, inorganically reduced sea-water and organically reduced sea-water. The abundance of carbonaceous sediments associated with exhalative ore deposits and the remarkable discoveries of an aphotic biota around hot-springs on the ocean-floor near the Galapagos Islands has enhanced the case for an organic reduction of sea-water. Sulphur isotope studies have usually indicated an inorganic source.

Such then was the agreement between experimental and field data that discovery of sulphides on the sea-floor was only a matter of time. Numerous examples of metalliferous sediments, of ferruginous and manganiferous crusts and indeed of active, but rather cool, springs have been reported in recent years. However the CYAMEX team have found the first sulphides. The technical problems of navigating and sampling in an oceanic rift are stupendous. The fact that the crew recognised the oddity of these rocks and sampled them is a credit to them and we can only look forward eagerly to results of their further investigations at the site.

But what exactly have they found? It would appear from their descriptions that they have, not a massive sulphide lens as such, but the deposits around

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the vents of hydrothermal springs. They have shown that sulphides can be formed directly on the sea-floor—that is, that sulphur is an integral part of the system and not a later, but necessary, additive The spires of sulphides and silica lie directly on pillow lavas and the fluids that were exhaled from these vents have deposited the bulk of their load elsewhere. Where? Were the fluids heavier than sea-water, in which case we can expect the imminent discovery of a genuine massive sulphide lens nearby; or lighter, their metals dispersed? These are the questions that must now be investigated. The chances of finding a massive sulphide lens are probably low—the system appears to have been oxidised too soon and too close to its vents, and many of its metals may never have been precipitated.

Finally, what of the economic implications? The recovery of massive sulphides from the sea-floor would seem to be economically remote with no shortages in terrestrial reserves of copper and zinc. Potentially cheap extraction of metalliferous brines offers a far better prospect. It is to this that the mining houses must turn their eyes. Is this discovery a complete fluke or could there really be a ribbon of sulphides along the ridges? Mid-oceanic ridges are generally far too exposed to the physical and chemical vagaries of the open oceans to have encouraged large scale, undisturbed, reduced environments for the common preservation of massive sulphides. Back arc basins, such as the Japan or Phillipine seas offer the same magmatic and hydrothermal processes, but in a far more protected environment. It is in these that a discovery of major sulphide deposits is more likely. □

The *recA* gene product

from B. A. Bridges

THE *recA*$^+$ gene in *Escherichia coli* is involved in many cellular functions; in particular it is absolutely required for general recombination (as distinct from site-specific or illegitimate recombination) It is also required for a number of processes which have been suggested to be 'inducible' by DNA damage. The prototype *recA*$^+$-dependent inducible process is induction of prophage λ and this is the only *recA*$^+$-dependent process for which a repressor (λ immunity repressor) is known and characterised. For others, such as Weigle reactivation, filamentation or control of DNA degradation, the existence of a repressor remains hypothetical. One thing that is clear, however, is that the *recA*$^+$ gene product is made in very large quantities following treatments (such as ultraviolet irradiation) which elicit prophage induction and other 'inducible' phenomena.

A recent breakthrough towards understanding the function of the *recA*$^+$ gene product came with the observation that a proteolytic activity capable of cleaving λ repressor was present in extracts of cells known to contain large quantities of a mutant *recA* protein specified by an allele of *recA* called *tif* (Roberts *et al. Proc. natn. Acad. Sci U.S.A.* **74**, 2283; 1977). Such cells constitutively express 'inducible' functions. Recently, the *recA* protein from *tif* cells has been further purified to chromatographic homogeneity and an ATP-dependent proteolytic activity specific for λ repressor has been shown to be associated with it (Roberts *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4714; 1978) *RecA*$^+$ protein possesses only about 20–25% of the proteolytic activity associated with the variant protein from *tif* cells.

The discovery of λ repressor inactivating activity associated with *recA*$^+$ protein has tended to focus attention on its possible role in controlling 'inducible' functions to the relative neglect of its role in genetic recombination Yet the earliest characterisation of the protein, which was only subsequently found to be the product of the *recA*$^+$ gene revealed that it bound to single-stranded DNA (Gudas & Pardee *J. molec. Biol.* **101**, 459; 1976) and there have been many observations suggesting that *recA*$^+$ protein at its normal relatively low level must interact directly with DNA during both recombination and repair, without any requirement for synthesis of large amounts such as might follow induction

Such a direct role of *recA*$^+$ protein in DNA metabolism has now been established. Following the report (Ogawa, *Cold Spring Harbor Symp. quant. Biol.* **43**, in press) that *recA*$^+$ protein hydrolyses ATP in the presence of single-stranded DNA, Weinstock, McEntee and Lehman (*Proc natn. Acad. Sci.* **76**, 126; 1979) have shown that *recA*$^+$ protein purified to near homogeneity catalyses an ATP-dependent renaturation of complementary single strands of DNA. The relative activities at different temperatures of the protein from a cold-sensitive *recA* mutant confirm that this activity is an intrinsic function of the *recA*$^+$ protein Thus *recA*$^+$ protein is a 'windase' or 'annealase' and as such can be readily envisaged to have a specific primary role both in recombination and in the repair of damaged DNA

In a parallel investigation following independent purification, Shibata, Das Gupta, Cunningham and Radding (*Proc natn. Acad. Sci U S.A.* in the press) have pursued the matter one step further and shown that the *recA*$^+$ protein can catalyse the ATP-dependent pairing of superhelical DNA and homologous single-stranded fragments to form D loops. Thus single-strand ends are aggressive and can invade homologous superhelical DNA in the presence of *recA*$^+$ protein; in its absence, such a reaction occurs only at high temperature. D loops are known to be a substrate for some endonucleases (including the *recBC* DNase). One can therefore begin to see how recombination may be initiated and the way now lies open for exploring the whole process *in vitro*.

In view of the involvement of the *recA*$^+$ gene in controlling degradation of damaged DNA and its possible involvement in allowing error-prone base insertion opposite a damaged template strand, it would also be interesting to investigate the effect of *recA*$^+$ protein *in vitro*, on say, exonuclease V activity and the 3′→5′ (proof-reading) exonuclease activity of DNA polymerase III. Three recombination-proficient alleles of the *recA*$^+$ gene, namely *tif*, *recA*$^+$ and *lexB* all yield a gene product with similar 'annealase' activity although they differ markedly in expression of inducible functions.

The data so far are consistent with *recA*$^+$ protein's being rather complex in its activities, apparently showing specific proteolytic activity, DNA-dependent ATPase activity and ATP-dependent 'annealase' activity. Moreover, it seems also to control its own induction and synthesis. Such complex activity in a simple tetramer of subunit molecular weight 40,000 is not inconceivable but neither is it common.

Because normal levels of *recA*$^+$ protein are insufficient to cause derepression of prophage λ one may well imagine that many phenomena depending on constitutive levels involve its acting as an annealase, for example normal recombination, post-replication recombinational repair, mutation in unreplicated DNA and repair of single-strand breaks produced by ionising radiation (this is not to say that raised levels of *recA*$^+$ protein might not enhance these processes). Phenomena that occur only with elevated induced levels of *recA*$^+$ protein could involve either annealase activity (for example, control of DNA degradation, multiple crossovers during Hfr x F$^-$ crosses (Lloyd *J. Bact* **134**, 929; 1978)) or re-

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pressor cleavage (λ induction for example) or possibly both. In some cases (Weigle reactivation, filamentation) the mechanism is still obscure.

There is still, however, a niggling doubt about ascribing the proteolytic activity to the *recA*$^+$ protein As Roberts *et al.* state, they cannot exclude the possibility that a contaminating protease is present in their preparations even though they are chromatographically homogeneous. Consistent with this possibility is the observation that bacteria containing constitutive high levels of *recA*$^+$ protein due to the presence of the controlling mutation *spr* do not show constitutive λ induction nor do crude extracts possess high ability to inactivate λ repressor (Roberts *et al Proc natn Acad Sci. U.S.A.* **75**, 4714; 1978) Several workers have suggested that the *recA*$^+$ protein requires a cofactor to be active in prophage induction. It is not totally inconceivable that such a cofactor might be the protease and that it is present in greater amounts in *tif* mutants (although such speculation does present its own difficulties) Thus although the evidence that *recA*$^+$ protein is both a protease and a DNA annealase may be sufficient to sustain the idea as a working hypothesis, more definitive evidence must be sought and the possibility of further revelations of the functions of this remarkable protein cannot be excluded. ☐

Poles apart?

from Clive Lloyd

THERE is still much to be learned about the mitotic apparatus, especially the nature of spindle organisers. Such microtubule-organising centres (MTOCs) are believed to exist at each spindle pole, where, by nucleating the assembly of microtubules, they help construct half-spindles along which sister chromatids are guided to opposite poles. *In vitro* studies confirm that microtubules can self-assemble, but the value of MTOCs is that they localise assembly and so help control planes of division important for shaping tissues. The most familiar MTOC is associated with the centriole which is a highly structured organelle resembling a vestigial flagellum. It is noteworthy, though, that amorphous pericentriolar material would seem to be the spindle organiser in *in vitro* polymerisation studies on Chinese hamster ovary cells so, if nothing else, the centriole provides a convenient signpost for such associated material (Gould & Borisy *J Cell Biol.* **73**, 601; 1977).

Definite pole bodies are to be seen in algae and fungi, but in higher plants we are confronted with the problem that such structures are not visualised in ultrastructural studies of dividing cells. This has forced the alternative view that accumulated endoplasmic reticulum seen at the poles could, by sequestering calcium, precipitate localised formation of microtubules (Hepler in *Mechanisms & Control of Cell Division* (eds Rost & Gifford) 1977), although it is admittedly difficult to see how, in the absence of a template, the number, pattern and precise location of microtubules could be controlled.

However, a recent study on the effect of a herbicide on 3T3 fibroblasts rekindles interest in presumptive spindle organisers since the drug is well known to produce mitotic abnormalities in plants. Oliver *et al.* (*Expl Cell Res.* **116**, 229; 1978), showed that chloro-isopropyl N-phenyl carbamate (CIPC) causes the formation of multinocleate cells by inducing multipolar mitotic spindles. *In vitro* polymerisation studies indicated that CIPC, unlike colchicine, did not interfere with tubulin assembly and so it was concluded that the herbicide had affected spindle MTOCs rather than microtubules *per se* This report on animal cells echoes the earlier study of Hepler and Jackson (*J. Cell Sci.* **5**, 727; 1969) on dividing nuclei in the endosperm of the African blood lilly. Whereas colchicine caused microtubule disassembly, IPC did not, but with the latter treatment multipolar spindles were formed. However, although microtubules were seen to radiate from some central locus, electron microscopy failed to give shape to the spindle pole. Following this, Coss & Pickett-Heaps (*J. Cell Biol.* **63**, 84; 1974) used IPC to examine division in the green alga, *Oedogonium cardiacum*. They, too, found that multinucleate cells were formed, suggesting that the herbicide increased the number of MTOCs No such bodies could be identified in thin sections but with high voltage electron microscopy on thick sections the picture was quite different, for morphologically distinctive spindle pole bodies were revealed in both normal and drug-treated mitoses This family of reactions on representatives of animal cells, higher plants and algae indicates that some divisible MTOC is being affected by the herbicides but it remains to be determined whether endoplasmic reticulum, as opposed to some other associated, perhaps amorphous, nucleation site, is being perturbed in higher plant cells ☐

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Chamber crystals in the stratosphere

from Brian A. Thrush

ONE aspect of stratospheric chemistry which has been largely overlooked in the recent flurry of activity has been the role of the aerosol particles These form the Junge layer in the lower stratosphere and have their peak density at an altitude of about 18 km. Although these particles, which have sizes in the range 0.05–1 μm, number only about one per cm^3, the amount of material they contain is comparable with the amounts of important trace materials in the lower stratosphere, such as the nitrogen oxides. If these species reacted efficiently with the aerosol particles, this process could be significant to the chemistry of the stratosphere. Chemical analysis shows this aerosol to contain ammonium sulphate, accompanied by persulphate and perhaps chloride, but recently Farlow *et al.* (*J geophys. Res.* **83**, 6207–11; 1978) have tentatively identified two nitrosyl sulphuric acids, $NOHSO_4$ and $NOHS_2O_7$ by electron diffraction studies of aerosol particles collected by U-2 aircraft.

The stratosphere is very dry, containing only a few parts per million of water, and it is interesting to note that $NOHSO_4$ is very stable under such conditions It is familiar to chemists as forming the 'chamber crystals' which appear in the lead chamber process for making sulphuric acid. They are formed from sulphur dioxide and nitrogen dioxide (NO_2) when insufficient water is present. The authors point out that if this compound comprised 20% of the sulphate in stratospheric aerosol then this would contain about one third of the amount of nitric oxide (NO) which is present at these altitudes Because the nitrogen oxides NO and NO_2 catalytically destroy ozone in the stratosphere, any process which affects their concentrations is potentially important In fact, neither the measured ratio of NO and NO_2 to nitric acid ($HONO_2$) nor the failure to detect pernitric acid (HO_2NO_2) in the lower stratosphere agree well with predictions based on known homogeneous chemical reactions. Reactions involving aerosol particles may be implicated here

In the stratosphere, these nitrogen compounds come mainly from nitrous oxide (N_2O) derived from denitrification processes and could therefore be

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enhanced by increased use of nitrate fertilisers. Some nitrogen oxides are directly injected into the stratosphere from the exhausts of high-flying aircraft. For this reason it is tempting to see the formation of 'chamber crystals' as a means by which the stratosphere can simultaneously purge itself of two pollutants, but any such contribution will be confined to the lower atmosphere (the troposphere). Settling of stratospheric aerosol would not provide a significant process for removing nitrogen oxides from the stratosphere, by comparison with rain out of nitric acid. Furthermore, the increase in stratospheric aerosol in recent years is generally attributed to volcanic eruptions such as Agung in 1963 rather than to the increased burning of fossil fuels because of the efficiency with which tropospheric processes remove sulphur oxides. □

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As an illustration of the general character of the woodcuts which so profusely adorn this volume [Cassell's Natural History, volume II] we have selected one of an interesting animal which has been a great puzzle to the systematic zoologist. "The Binturong (*Arctitis binturong*) is a curious little animal of a black colour, with a white border to its ears; it has a large head and turned-up nose; its tail is immensely long, thick and tapering, and which is very remarkable it is prehensile, like that of a new world monkey. It is quite nocturnal, solitary and arboreal in its habits. Its howl is loud. It is placed by Mr Parker among the group of the civets."
From *Nature* **19**, 13 Feb. 345; 1879.

The spiral structure of galaxies

from Bernard Jones and Scott Tremaine

ONE of the most vexing problems in theoretical astrophysics has been the explanation of the beautiful spiral patterns seen in many galaxies. It is easy enough to produce spiral structure in both galaxies and coffee cups: because they rotate differentially (the inner parts revolve faster than the outer parts), an irregular gas cloud or a dribble of cream is rapidly sheared into a spiral feature which looks very similar to a galactic spiral arm. The difficulty is that by now most galaxies have undergone 50–100 revolutions, so that any primordial spiral arms would have been wound up beyond recognition.

The most likely resolution of the winding problem for differentially rotating galaxies is that the spiral arms are not material objects, but waves of gravitational potential which propagate around the galaxy. As the interstellar gas passes through the wave it is shocked and forms bright blue stars which we see as a spiral arm; the spiral arm appears narrow because the lifetime of the stars is short compared to the time the wave needs to propagate around the galaxy. This part of the theory is comparatively well understood, but understanding the origin and persistence of the underlying wave pattern in the gravitational potential has been surprisingly difficult.

Most of the work in the past 15 years has been directed towards explaining the wave as a normal mode of the galaxy, like the oscillations of a drumhead. One difficulty with this picture is that the oscillations are damped by the very shocks that make the spiral arms visible, and the damping time for the normal mode is much less than a typical galactic lifetime. Another problem is that observations show that spiral arms are always oriented in the same sense: their convex sides face in the direction of galactic rotation. This clue tells us that galaxies are not time-reversal invariant, because time-reversal would change the direction of galactic rotation. A neutral oscillation is time-reversal invariant and a theory based on neutral oscillations therefore cannot explain this observation.

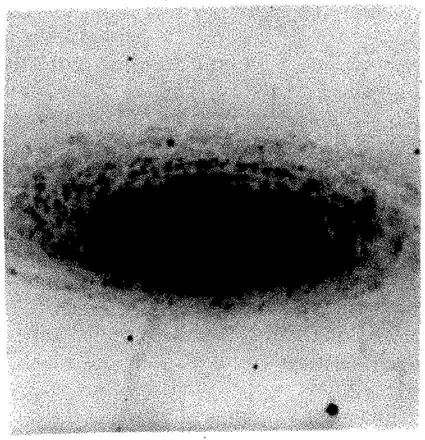
The difficulties with the simple normal mode theory for the origin of spiral structure have stimulated many important dynamical studies of galaxies. What has emerged is the realisation that differentially rotating galaxies, like the diaphragm of a drum, need to be continually excited to maintain their vibrations. A galaxy that is not excited in some way loses its grand spiral structure very quickly. Two kinds of "drumstick" have been proposed: either a satellite galaxy or a central bar-like structure such as is seen in many galaxies could provide a perturbing potential which would excite strong spiral waves.

J. Kormendy and C. Norman are the first astronomers to confront these theoretical ideas with a systematic and careful consideration of observational data; (*Astrophys. J.*, in the press). They collected pictures of all spiral galaxies whose rotation curves were known. Their sample contained 69 galaxies, of which 15 had to be discarded either because they appeared edge-on and the spiral pattern was invisible or they were too irregular to classify. Of the remaining 54 galaxies, 25 had bars and 8 had close satellite galaxies and as the theory would predict, the presence of such objects was always accompanied by well-defined large scale spiral arms.

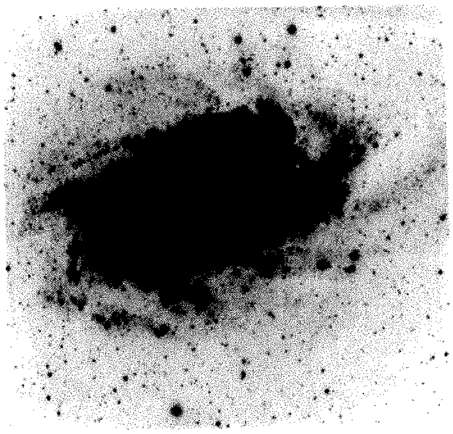
The most interesting galaxies are the remaining 21; they apparently have no known way of sustaining a spiral wave. Kormendy and Norman found that in every case, either the spiral arms were short and ill-defined, or the galaxy rotated uniformly in the region where the arms were prominent.

Retrospectively, it is easy to understand this result. In the case where the region occupied by the spiral arms rotates uniformly, there is no winding problem and the spiral structure is

NGC2841



M33



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relatively easy to maintain. In a region of strong differential rotation, the vibrations are sheared into small scale spiral structure. The difficulty in spiral structure theory has always been to explain the galaxies which rotate differentially yet show well organised long spiral arms. What Kormendy and Norman have argued is that it is just these galaxies that are always accompanied by a bar or a companion

Two figures taken from their paper serve to illustrate their general conclusion. NGC 2841 is an archetype of those galaxies that have neither bar nor companion and have no coherent spiral pattern. This is to be contrasted with M33 which has neither bar nor companion, yet has a clearly identifiable spiral design contained within the region of the galaxy where there is almost solid-body rotation The region of solid-body rotation in NGC 2841 is much smaller in relation to the size of the visible disk. What we may be seeing now is the emergence of a galaxy classification scheme that can be tied directly to galactic dynamics. ☐

T-cell regulation

from Rod Langman

DISCUSSIONS at the most recent Armand Hammer Workshop* centred on suppression and suppressor factors elaborated by T lymphocytes, Ir-gene effects and H-2 restriction, and on the question of idiotypic determinants on T-cell factors and T-cell receptors An impressive array of data was closely scrutinised; however, there was little discussion of how this data could be interpreted in terms of the current theories of immunology. The calm of hindsight has revealed some concepts and details that must be settled before we are in a position to manipulate the immune system rationally.

The network concept of an immune system has captured the imagination of many immunologists, and while associative recognition theories of immune regulation are less popular, they seem to fit many of the facts better. What, then, are the critical differences between these two viewpoints? First, the network demands a system of cell–cell activation and inhibition interactions that are balanced, and which exist independent of antigen. Associative recognition theories require cell-cell interactions to occur only by way of antigen bridges which signal activation, leaving suppression with a modulating role during antigen-induced activation Second, the network demands that suppression be paramount in the discrimination between self and non-self (which leads to unresponsiveness towards self antigens) while in the associative antigen recognition theories, unresponsiveness to self is a case of T-cell deletion that involves mechanisms other than suppression.

With these distinctions drawn between the two conceptual approaches to immunology, what experiments are most likely to resolve the differences? On the question of self-tolerance the radiation chimaeras will probably prove the most informative. For example, consider a mouse of strain A which has been thymectomised, irradiated, restored with fetal liver (AT × FL, for short) and grafted with an $(A \times B)F_1$ thymus. When this mouse is immunologically mature, say 8–12 weeks post restoration, removal of the F_1 thymus graft will (1) remove the tolerated B antigen, and (2) prohibit any further T-cell maturation If the suppressionists and network theorists are right then reactivity to B should be readily recovered; whereas, if the opposition is correct, reactivity to B will not be recoverable.

Another aspect of the self and non-self distinction deals with the phenomenon of H-2 restriction. Reduced to its simplest components in the cytotoxic T lymphocytes (CTL), overall specificity of effector cells is determined by two different antigen sets; one encoded within H-2K or H-2D (restriction antigens), the other encoded elsewhere, either by viruses or other genes such as H-Y (X antigens). The restriction (R) antigens are essentially within the 'self'-domain, whereas the X antigens are within the 'non-self'-domain There was some discussion as to whether CTL recognised R and X antigens separately or only a new antigenic determinant (NAD) formed by the complexing of R and X antigens. However, M. Cohn (Salk Institute) argued that restriction specificities (that is, anti-K/D) are selected in the absence of X (that is, non-self) antigens (as shown by Bevan and Zinkernagel's early chimaera experiments) and therefore NAD's cannot be the only class of antigens recognisable by CTL. Thus, it seems difficult to avoid having two antigen recognition sites on the T cell, one for self (anti-R), the other for non-self (anti-X), and from this it follows that each site must carry different idiotypic determinants. However, if each CTL carries two different idiotypic determinants, then each CTL will have conflicting inputs from within the idiotype-regulated network. In short, the best available interpretation of H-2 restriction is simply incompatible with network theories. As a consequence, believers in the network insist that H-2 restriction must be a complicated form of suppression; but nobody has proposed a mechanism.

Despite the demise of the network there remains a body of data showing that idiotypic recognition can be involved in the regulation of immune responses. Within the framework of associative recognition, idiotypic recognition, and even idiotypic specific regulation, can certainly occur, but it must be a second-order phenomenon, not primary as the network theorists believe.

Restrictive recognition can be readily incorporated into associative antigen recognition theories, but this opens the question as to the origin of the receptors for restricting and X antigens. There is a growing body of evidence that receptors on some classes of T cell have idiotypic specificities which parallel those found on B-cell derived immunoglobulins, and these idiotypes are apparently controlled by genes mapping in V_H. Oddly enough, the immunoglobulin V_H controlled idiotypes usually require V_L for expression of the idiotype, while in T cells there is no evidence for an L chain related to the Ig set (that is, κ or λ). The receptors bearing NP-idiotype isolated from T cells (K. Rajewsky, Köln), and the ARS and GAT-idiotypes found on T-suppressor factor (B. Benacerraf, Harvard University) have been serologically and chemically analysed and provide strong evidence that at least some T cells have receptors which carry idiotypes controlled by V_H genes. It is important to question the significance of these findings in terms of the role of these idiotype-bearing T cells in regulating normal immune responses One is left to ponder quietly the fact that it is only anti-idiotypic antisera that put V_H-determined idiotypes on T cells This is especially worrisome in light of the known mutations which affect T-cell responses in an antigen-specific way; without exception, they are not V_H linked. Are the anti-idiotypic antisera leading us down the right or wrong pathway? Perhaps it is too soon to judge, but on purely theoretical and aesthetic grounds the T-cell receptor is unlikely to be encoded in the V_H locus; the H-2I(JEC) region offers intriguing possibilities.

The phenomenon of Ir genes, particularly those mapping to the H-2IA and H-2I(JEC) regions which show complementation, has continued to fascinate. If there is any consensus among Ir-gene speculators, the macrophage or antigen presentation system seems the most likely site of Ir-gene action. Perhaps the best support for the macrophage–Ir relationship came from experiments based on an antigen-

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*An Armand Hammer Workshop on the problems of T-cell regulatory mechanisms was held at The Salk Institute, San Diego on 26–30 November, 1978

Rod Langman is at the Salk Institute, San Diego driven, macrophage and T-cell dependent, proliferative assay (PETLS), which showed that a mixture of two complementing non-responder macrophages were able to complement in the stimulation of F_1 T cells while of course the F_1 macrophage did stimulate (W. Paul, National Institutes of Health, Bethesda). This problem might be clarified by the use of two chimaeras. For example, if A and B are two complementing non-responders, the following two chimaeras would test complementation effects: (1) an (A × B)F_1 is grafted with A and B thymuses separately so that F_1 macrophages present antigen to a mixture of A and B restricted cells; and (2) an (A × B)F_1 mouse is irradiated and reconstituted with a 50:50 mixture of A + B cells which will then differentiate in an F_1 thymic epithelial environment so that each cell, T and macrophage, is genetically either A or B but is restricted to F_1 antigens. The results from these two chimaeras will distinguish between intracellular complementation and intercellular complementation.

Some Ir-1 gene phenomena are closely tied to restricting antigens, H-2K, IA and D, while others are linked to the H-2I(JEC) region which is not known to code for any restricting antigens. Although from the known genetics and structure of immunoglobulins it is expected that V_H and V_L should complement in generating unique antigen-binding specificities, it is rarely suggested that Ir-A and Ir-(JEC) gene complementation signify interactions of V_L–V_H type. However, a combination of serology and chemistry has shown that there are molecular, and possibly antigenic, complexes formed between gene products from the I-A and I-(JEC) regions (Uhr, Southwestern University; H. O McDevitt, Stanford University). Another intriguing possibility is that I(JEC) encodes the V region of the T-cell receptor. Thus, after 15 years, we still seem to be grasping for a conceptual framework that converts the phenomena of Ir genes into commonsense understanding. In fact, this is a fair comment on T-cell regulation in general. ☐

Confining current in tokamaks

from J. G. Cordey

THE poloidal magnetic field in a tokamak fusion reactor, which is essential for plasma confinement, is sustained by a toroidal current In the present experiments this current is driven by an electric field which is produced inductively by arranging for the toroidal plasma to be the secondary of a transformer. This technique of generating the toroidal current is inherently pulsed and since it seems that for a reactor to be economic it will have to be run in a steady state mode, some alternative steady state method of generating the current will have to be found.

The first such scheme was put forward by Ohkawa (*Nucl. Fusion* **10**, 185; 1970), his suggestion was to inject a separate species of fast ions in the direction parallel to the field line so that these ions circulate around the torus and form a toroidal current. The strength of the current is proportional to the number of transits the fast ions make around the torus before becoming thermalised This number is referred to as the stacking factor S and equals $\tau_s V_f/2\pi R$ where τ_s is the fast ion slowing down time, V_f is the mean velocity of the fast ions, R is the major radius of the torus Thus the total fast ion current is I_0S where I_0 is the injection current

The electrons gain momentum from the fast ions through Coulomb collisions, so they also have a net drift velocity in the same direction as the fast ions and hence tend to reduce the total current. The expression for the net current which is obtained by balancing the forces acting on the electrons may be put in the form $J=I_0S(1-Z_f/Z_{eff})$ where S is the stacking factor, Z_f the charge number of the fast ions, and Z_{eff} the effective charge number of the plasma. Thus it would appear at first sight that unless the plasma charge and fast ion charge numbers are different there would be no net current. However, if the effects of the trapping of electrons in the toroidal field gradient and the non-Maxwellian nature of the electron distribution are taken into account, there is a net current, even when $Z_f=Z_{eff}$.

The first measurement of this beam-induced current was made on the Culham Levitron (Start *et al Phys. Rev. Lett.* **40**, 1497; 1978) In this experiment a modulated beam of fast protons was injected into the device and the net circulating current was obtained from the voltage induced in a pickup coil which looped the plasma. The current was measured as a function of electron temperature and found to differ significantly from the predictions of the simple theory The discrepancy was due to the inadequacy of the theoretical model and a kinetic treatment (Cordey *et al. Culham report CLM—P547*; 1978) which took into account the non-Maxwellian nature of the electron distribution and electron-electron collisions was found to give much closer agreement. The implications of the new theory for tokamaks are fairly minor since the correction term is of the order $\bar{v}_f^2/v_e^2$ (where v_e is the thermal velocity of the electrons), which is usually small in tokamaks. The full expression for the beam-induced current in a tokamak including trapped electron effects (Connor & Cordey *Nucl. Fusion* **14**, 185; 1975) and the above correction

$$J=I_0\,S\left\{1-Z_f/Z_{eff}+1.46\,\varepsilon^{\frac{1}{2}}\frac{Z_f}{Z_{eff}}\;A(Z_{eff})-1.2\,\frac{\bar{v}_f}{v_e}^{2}\right\}$$

where $A(Z_{eff})=1.68$ for $Z_{eff}=1$; 1.18 for $Z_{eff}=4$ and 1 for $Z_{eff}=\infty$. There has been no clear identification of this current in a tokamak yet. However, sufficiently high neutral injection power is now available on several experiments and so one expects an observation of this current in the near future.

Another method of generating the toroidal current has recently been proposed by Fisch (*Phys. Rev Lett.* **41**, 873; 1978) In this scheme a small group of high energy electrons are accelerated in a direction parallel to the field lines by high phase velocity r.f. waves. The frequency of the waves is near the lower hybrid frequency and they are launched from an "end fire waveguide" array, which directs the power flow parallel to the field lines. The waves transfer their momentum to the electrons through resonant particle damping and this drives a current in the plasma.

The power required to drive the current is calculated from the power dissipated by the group of resonant electrons through collisional drag on the remainder of the plasma An effective r.f. resistivity η_{rf} is then defined and this may be compared with the ohmic resistivity η_{ohmic} of the plasma. For equal currents the ratio of $\eta_{rf}/\eta_{ohmic}=10\,v_e^3/v_1^2\,v_D$ where v_D is the drift velocity of the electrons in the ohmic case and v_1 is the velocity of the resonant electrons in the r.f. case. Thus for low current levels (v_D small) the r.f. current dissipates more power than the ohmic current. At higher current levels, provided $v_1 \gg v_e$ the r.f. current begins to dissipate less power than the ohmic current.

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Calculations are given for reactor parameters, and it is found that the power required to drive the current is approximately 3% of the total fusion power output and that only 1% of the area of the wall would be required for the wave guides.

Clearly an experimental demonstration of both the fast ion and r.f. schemes in a tokamak is required before any realistic assessment of the reactor potential of either of these schemes can be made. ☐

Masses of exotic nuclei

from John Edgington

THE stable nuclei occupy a narrow strip on a plot of proton number Z versus neutron number N. Increase Z or N by a few units, and the resulting nuclei undergo β-decay, increase Z or N greatly and even this limited stability disappears, the excess nucleons being ejected singly or in clusters

This fragility of all but a limited number of nuclei has to be explained, eventually, in terms of the individual nucleon-nucleon interactions. Given the complexity of many-body calculations, it is hardly surprising that for practical purposes, such as estimating nuclear binding energies and masses, simpler prescriptions are used. These 'semi-empirical' formulae are based on the liquid-drop model but include the main features of the individual interactions in an approximate way. The values of the numerical coefficients involved are adjusted to fit the masses of stable nuclei, and the most widely used of these formulae, the Garvey-Kelson relation (Garvey *et al.*, *Rev. Mod. Phys.* **41**, Sl; 1969) also considers correlations between nuclei with closely similar values of Z and N.

The most stringent tests of these formulae occur, of course, for nuclei far from the narrow stability strip. Since these are very short-lived, some subtle experimental techniques are needed. A fine example is that reported from the LAMPF accelerator, Los Alamos, by a LASL/Northwestern University group (Seth *et al.*, *Phys. Rev. Lett.* **41**, 1589; 1978) who have measured the mass of ^{18}C This exotic nuclide, with twice as many neutrons as protons is one of a class of nuclei having N = Z + 6 which seem to mark a significant boundary on the stability map of light nuclei. If N and Z are both odd, these nuclei are unstable to particle emission; if even, they appear to be stable. Like other neutron-rich nuclei, ^{18}C can be produced fairly readily in heavy ion collisions, or as a spallation product of heavy nuclei, but these violent processes are quite unsuited to precise mass measurements.

Seth and his colleagues have produced ^{18}C by the double charge exchange (DCX) reaction $^{18}O(\pi^-,\pi^+)^{18}C$, using the energetic pion spectrometer at LAMPF and measuring the energy of both the incident π^- and the outgoing π^+, to obtain the binding energy of ^{18}C by subtraction The technical difficulties are great. There is a huge background of electrons from π° production and decay, but these are entirely eliminated by time-of-flight and Cerenkov counter techniques; also, the spectrometer's energy resolution is too poor for mass measurements, so a calibration reaction involving nuclei with known masses, $^{12}C(\pi^-,\pi^+)^{12}Be$, was also measured. The group had previously confirmed (LA-7457-PR Progress Report, Sept. 1978) that their equipment had the desired sensitivity, using the simpler reaction $^{9}Be(\pi^+,\pi^-)^{9}C$, since a target of natural beryllium metal poses fewer difficulties than the enriched ice target used for the ^{18}O DCX reaction.

Seth *et al* compared their result for the mass excess of ^{18}C, 24.91 ± 0.15 MeV, with the Garvey-Kelson relation. Earlier predictions had suggested a somewhat lower binding energy, but a recalculation of the G–K prediction, using recent measurements of the ^{17}C and ^{18}N, yielded a mass excess of 25.04 ± 0.10 MeV, in excellent agreement.

It is worth stressing that Seth *et al 's* measurement would not have been thought possible, even with technical ingenuity, two years ago when the cross sections for the DCX reactions (π^-,π^+) were thought to be exceedingly small. Conventional wisdom had it that reactions connecting analogue nuclear states were much more likely than those between non-analogue states. Roughly, analogue states are corresponding states in different nuclei which differ only in that one or more protons and neutrons have been interchanged, but no rearrangement of the nucleon orbitals has occurred. According to this opinion, any necessary orbital rearrangement would depress the cross section greatly. In 1977 another group working at LAMPF, B. M Preedom's LASL/University of South Carolina/ANL collaboration, showed that the reaction $^{18}O(\pi^+,\pi^-)^{18}Ne$, connecting analogue states in the two nuclei, had a cross section only about twice as large as that of $^{18}O(\pi^+,\pi-)^{18}Ne$. The latter converts neutrons in a filled shell to protons occupying the next higher orbital, and so connects non-analogue states. Now, whereas it is always possible to find nuclei with excess neutrons on which the (π^+,π^-) reaction can connect analogue states with no orbital rearrangement, there are no stable nuclei (other than ^{1}H and ^{3}He) with excess protons; and so (π^-,π^+) reactions necessarily connect non-analogue states.

The results of Preedom's group (*Phys. Rev. Lett* **38**, 149; 1977; *Phys. Lett* **69A**, 55; 1977) greatly encouraged study of (π^-,π^+) reactions, since there is now seen to be no reason to suppose that their cross sections are unusally small. Even so, Seth *et al.* observed only about twenty events leading to the ground state of ^{18}C. They now plan to measure the mass of several more extremely neutron-rich nuclei, including ^{48}Ar which has Z = 18, N = 30; this will be reached by the reaction $^{48}Ca(\pi^-,\pi^+)^{48}Ar$. We may expect other such measurements in the near future, especially as the re-evaluated Garvey-Kelson relation now predicts greater stability against particle emission than before. ☐

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The current state of eukaryotic messenger RNA

from H R. V. Arnstein

THE isolation of different messenger RNAs and their translation into specific proteins in a number of cell-free systems are now almost routine. Moreover, the nucleotide sequences of several messengers have been established Given that the concept of mRNA emerged less than 20 years ago impressive progress has been made

At a recent EMBO Workshop* the main topics were heterogeneous nuclear RNA (hnRNA) processing, hnRNA–protein complexes, free cytoplasmic messenger ribonucleoproteins (mRNPs), the cytoskeleton in relation to protein biosynthesis, polysomal mRNA–protein complexes and translational control

The general picture which emerged was that the molecular aspects of transcription and the subsequent processing of the primary transcripts are known in outline, but there is still considerable scope for detailed studies of the enzymes involved and of the control of mRNA synthesis in eukaryotes Moreover almost nothing is known about the transport of processed mRNA from the nucleus into the cytoplasm and there is uncertainty about the proteins which occur in association with mRNA precursors in the nucleus and with free and polysomal mRNA in the cytoplasm. These mRNA–protein complexes are a characteristic feature of eukaryotic cells, but their functional significance is obscure

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*Held at Hapert, The Netherlands on 27 November–1 December, 1978 The meeting was organised by H Bloemendal and W J. van Venrooij of the Biochemistry Department, University of Nijmegen

As discussed by J. E. Darnell (Rockefeller University) for adenovirus mRNA, capping of transcripts takes place very early, perhaps at the beginning of transcription RNA polymerase reads past the site at which poly(A) is subsequently added and the primary transcript is cleaved endonucleolytically before addition of the 200-residue poly(A) tail. Methylation then takes place, followed by splicing to give the final processed transcript. The size of the primary transcripts of eukaryotic genes remains somewhat controversial with K. Scherrer (Cancer Institute, Lausanne) still favouring very large products. This question will ultimately be settled by comparisons of nucleotide sequences of genomic DNA, primary transcripts and processed messengers The use of restriction endonucleases and DNA cloning has led to rapid progress as illustrated by the discussions of globin gene expression (R. Flavell, University of Amsterdam), the structure of late SV40 mRNAs (W. Fiers, University of Ghent), post-transcriptional processing of the yeast tyrosine tRNA, which contains a 14 nucleotide intron (J. S. Beckmann, Beth-Dagan, Israel), and mouse pre-mRNA structure (A. P. Ryskov, Inst. Molecular Biology, Moscow).

The complexity of heterogeneous nuclear RNA was illustrated by the isolation from nuclei of both 30–50S monomers and heterogeneous polymers, which differ in protein composition and resistance to ribonuclease digestion (M. Jacob). In some cells, the 30S nuclear RNP contains multiple copies of four major structural polypeptides (molecular weight 34,000–40,000) held together by protein-protein interactions (T E. Martin, University of Chicago), whereas in others as many as 87 different proteins have been identified, including some 20 with molecular weights of 30,000–40,000 (Scherrer) In addition to hnRNA, nuclear RNP particles contain also a low molecular weight RNA (snRNA) which has a longer half-life and appears to be hydrogen-bonded to hnRNA by sequences of some 15–25 nucleotides (A. Alonso, Cancer Centre, Heidelberg).

Free and polysomal messenger ribonucleoproteins present in a wide range of different cells were much discussed. No definite conclusion emerged concerning relationships between any particular protein(s) and the functional state of the messenger, but there is increasing evidence for the view that mRNP proteins may be turning over independently of the mRNA. Thus, proteins associated with free mRNP from muscle cells were reported to be displaced during translation of the messenger in reticulocyte lysates (S. Sarkar, Boston Med. Res. Inst.). The widespread occurrence of RNA-binding proteins in eukaryotic cells (L. P. Ovchinnikov, Molecular Biology, Moscow), as well as the observation that free globin mRNA becomes associated with proteins during translation in a cell-free system from Ehrlich ascites tumour cells (H R V Arnstein, King's College, London), all lend support to the idea of a dynamic state of mRNP proteins despite the well-known strong chemical interaction between the proteins and mRNA

Differences in mRNP proteins of membrane-bound and free polysomes (N. Standart, Univ. College, Cardiff) and also after virus infection of cells (J G Tasseron-de Jong, University of Leiden) suggest possible functions of proteins in relation to the intracellular location of messengers and control of translation Two other specific proteins, ribophorin I and II, which are present in the rough endoplasmic reticulum of rat liver and other secretory tissues are also involved in binding ribosomes to the membrane (D. A. Sabatini, New York University Medical Center).

Several contributions dealt with the cytoskeleton and its significance for protein synthesis. Thus, polysomal mRNA may be linked to the cytoskeleton by the poly(A) tail and poliovirus mRNA displaces cellular mRNA from intermediate filaments, which may explain the cytopathic effect of poliovirus and other viral infections (S. Penman, Massachusetts Institute of Technology). In lens tissue a particular membrane protein is synthesised by polyribosomes which are specifically bound to the action of the cytoskeletal framework (H. Bloemendal, University of Nijmegen). It was also suggested (A. O. Pogo, N Y. Blood Center) that in interphase hnRNA is bound to a highly ordered structure formed by non-histone protein interactions and that after post-transcriptional processing mRNA leaves the nucleus already attached to the cytoskeleton. Thus, both processing and transport of mRNA may be dependent on the internal structural organisation of the cell.

In the session on translational control, differences in the distribution of mRNA between the polysomal and non-polysomal cytoplasmic fraction were reported in Friend erythroleukaemic cells before and after induction of globin synthesis with dimethylsulphoxide (F. Gabrielli, University of Pisa) and in mouse ascites cells (Martin). Also, during the development of the dormant gastrulae of *Artemia salina* changes seem to occur in both the translational efficiency and sequence complexity of mRNA (T C. James, NIMR, London). Dormant gastrulae contain a unique 19S protein complex, which is an aggregate of 40–50 molecules of a 24,000 molecular weight protein with similar properties to the *Artemia* elongation factor–1$_H$ (M Kondo, University of Antwerp). On resumption of development this complex decreases, suggesting the existence of translational control involving elongation either in addition to or instead of the more usual regulation of initiation. Finally, the apparently endless complexities of translational control were illustrated by the discovery of an 11S cytoplasmic protein which prevents the inhibition of protein synthesis by the haemin-controlled repressor (H. O Voorma, University of Utrecht). This factor is also able to reactivate reticulocyte lysates after preincubation in the absence of haemin, has no eIF$_2$ activity and probably functions by restoring the activity of the endogenous initiation factor. □

A hundred years ago

CAPTAIN COOK

IT seems on first thoughts rather a strange proceeding to publicly celebrate the centenary of the death of a great man, especially when that death was a murder. But this is what the Paris Geographical Society have arranged to do tomorrow in the case, not of any of their own explorers or navigators, but in the case of England's greatest exploring navigator, Captain James Cook, who was murdered 100 years ago tomorrow by the natives of the Sandwich Islands. Cook, and with him England, owed some gratitude to the French, whose government of the time, though at war with this country, generously gave instructions to their warships and colonial governors, not only not to molest Cook in his pursuit of knowledge, but to render him all reasonable assistance. To him we owe the discovery of the Sandwich, and many other Pacific Islands . . and proved that [Australia] was unconnected with New Guinea, and above all, dispelled the long-lived illusion of a great southern continent, having been the first to cross the Antarctic Circle.

From *Nature* **19**, 13 Feb., 334; 1879.

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articles

Thermal–timing instability in neutron stars

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A thermal instability can occur in neutron stars, in which a significant burst of heat is generated in a short period of time. Accompanying this heat pulse is a change in the pulse repitition rate of the pulsar. A perturbation analysis of a simple model shows that the instability becomes easier to trigger as the star's initial temperature is reduced, and that once started it can grow very rapidly. The resulting time evolution of the pulsar angular velocity may resemble a period jump.

IT has been known for some time that, if neutron star interiors are superfluid, heat will be frictionally dissipated within them as their rotation slows. It is shown here that in certain circumstances this process can become unstable: that the frictional generation of heat can undergo a runaway, depositing a significant burst of heat in the star in a short period of time.

This runaway is purely thermal in nature: it results from an instability in an energy-generation process. Nevertheless, it has observable effects on the rotation rate of the pulsar. Neutron star interiors rotate more rapidly than their surfaces, and the frictional coupling between the two increases with increasing temperature. A burst of heat therefore tends to cause the star to 'seize' up: the inside slows down, and the outside—the pulsar—speeds up. The thermal instability manifests itself through its pulsar timing effects.

The obvious question is whether such a process has anything to do either with the well-known period jumps that have been observed in the Crab and Vela pulsars, or with the restless behaviour which apparently occurs in all pulsars. This possibility is considered later. However, a less obvious but equally important question, and one that is not considered here is: if the steady-state rotation of a neutron star can become unstable, what is the rotational state of such a star?

The instability in words

Baym *et al.*[1] state that a neutron star consists of a superfluid component and a charged-particle component; the pulsar clock is determined by the rotatiom of the charges (see ref. 2 for a more detailed review). The two components are coupled together by a frictional force density **F**:

$$\mathbf{F} = \rho_s \rho_c \mathbf{v}/\rho\tau \tag{1}$$

where ρ_s is the mass density of the superfluid component, ρ_c that of the charged component, $\rho = \rho_s + \rho_c$ is the total density, **v** the relative velocity difference between the two components and τ the relaxation time describing interactions between the two. The force (**F**) slows the rotation of the superfluid. In the steady state the relative velocity difference **v** adjusts itself until **F** is sufficient to slow the superfluid at the same rate as the charges. This slowing down, because it is caused by friction, generates heat at a rate $\mathbf{F}\cdot\mathbf{v}$ erg cm^{-3} s^{-1} throughout the superfluid.

The star also loses thermal energy through radiation (of photons from its surface, for instance). If C is the specific heat of the star as a whole the equation for its temperature T will have the form

$$C\dot{T} = \int \mathbf{F}\cdot\mathbf{v}\, \mathrm{d}^3r - \{\text{cooling rate}\} \tag{2}$$

Of the quantities entering into the first term on the right-hand side of equation (2) only the relaxation time τ depends on the temperature: τ decreases as T increases (see equation (5)). Therefore, this heating term is an increasing function of T. The same is true of the cooling term. There are only two possibilities: either the heating term increases with temperature faster than the cooling term, or it does not. These two cases are sketched in Fig. 1.

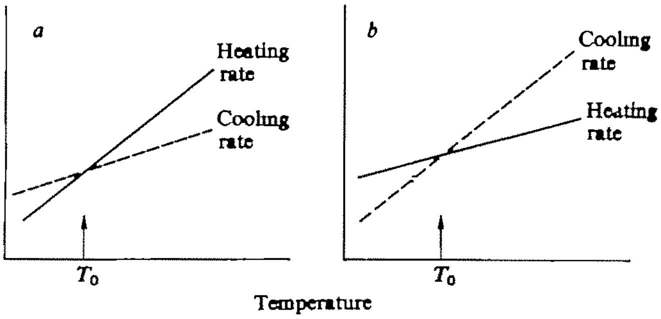

Fig. 1 The dependence on temperature of the heating term (solid lines) and cooling term (dashed lines) of equation (2). The time rate of change of the temperature is determined by their difference. T_0 denotes the steady-state temperature. *a*, Unstable; *b*, stable.

Figure 1 determines the time rate of change of T using the difference between the heating and cooling terms. A time-independent temperature, T_0, is obtained if they are set equal. This is the steady-state frictionally generated temperature of the star.

Is this equilibrium configuration stable? If we prepare a star with temperature, T_0, and then perturb the temperature slightly, then Fig. 1 shows that in (*a*) the perturbation increases in time, while in (*b*) it dies out. Case (*a*) is thermally unstable, both against sudden heatings and sudden coolings. Case (*b*) is stable. A star will be thermally unstable if its energy-generation mechanism is more sensitive to the temperature than is its cooling mechanism.

It is not known whether vortex lines pin to nuclei in neutron star crusts. If they do, a turbulent boundary layer may result, in which the processes described above should take place. However, no quantitative theory of this layer is available.

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Perturbation theory on a simple model

Most of the essential features of the instability can be obtained quite easily from the simple two-component model introduced by Baym *et al.*[1] in which the superfluid is supposed to be rotating rigidly. The known pulsars are cold enough for the dominant mode of cooling to be photon emission from the surface. Confining our attention to this case, and combining equations (1) and (2) we obtain after an obvious transformation:

$$C\dot{T} = \frac{I_s I_c}{I\tau}[\omega - \Omega]^2 - 4\pi R^2 \sigma T_e^4 \tag{3}$$

where I_s is the moment of inertia of the superfluid, I_c that of the star, $I = I_s + I_c$, ω is the superfluid angular surface velocity and Ω that of the charges, and T_e is the surface temperature of the star. Here we will take T_e to be proportional to T and neglect the temperature dependence of the specific heat: these assumptions are for convenience only and do not affect the qualitative features of the instability. Equation (3) is then of the form

$$\dot{T} = a/\tau(T) - bT^4 \tag{4}$$

with the factors a and b independent of temperature. (In a perturbation a will change because ω and Ω change. This, however, is a second order effect.)

Consider a different problem: simple cooling from the steady-state temperature with the energy generation mechanism turned off. From equation (4) we find the cooling time $t_{\text{cool}} \equiv (T/\dot{T})_0 = 1/bT_0^3$. This cooling time is of the order of thousands to millions of years for the known pulsars[3]. The steady-state condition $\dot{T}_0 = 0$ then yields $a/\tau_0 = bT_0^4 = T_0/t_{\text{cool}}$.

The relaxation time τ has been discussed by Feibelman[4] and Harding *et al.*[5]. Here we adopt Feibelman's result, which can be written

$$\tau = (A/T)\exp\{T_a/T\} \tag{5}$$

In equation (5) A is a complicated function of density only, and T_a is the activation temperature for thermal creation of a vortex core excitation: $T_a = \Delta^2/\varepsilon_f k$ where Δ is the superfluid energy gap, ε_f the neutron Fermi energy and k the Boltzmann constant.

Now do perturbation theory about the equilibrium temperature T_0. Writing

$$T(t) = T_0[1 + \varepsilon(t)] \tag{6}$$

we obtain from equations (4–6) and the above relationships the perturbation equation for the temperature to first order in ε:

$$\dot{\varepsilon} = \{[1+\varepsilon]\exp[\varepsilon T_a/T_0] - [1+4\varepsilon]\}/t_{\text{cool}} \tag{7}$$

For infinitesimal perturbations we expand the exponential to find

$$\dot{\varepsilon} = \frac{\varepsilon}{t_{\text{cool}}}\left[\frac{T_a}{T_0} - 3\right] \tag{8}$$

In an instability $\dot{\varepsilon}$ would have the same sign as ε: hot stars are stable, but when the steady-state temperature drops below 1/3rd of the activation temperature the star becomes unstable, both relative to heating and to cooling. The growth time of the instability, however, is of the order of t_{cool}, which is too long to be interesting. Thus the star is essentially stable against infinitesimal perturbations for all temperatures.

The same is true for finite cooling perturbations ($\varepsilon < 0$). The case of a finite heating perturbation at low temperatures, however, is quite different. With $0 < \varepsilon \ll 1$ but $T_0 \ll T_a$ we obtain from equation (7)

$$\dot{\varepsilon} = t_{\text{cool}}^{-1}\exp(\varepsilon T_a/T_0) \tag{9}$$

in which the exponential can be much greater than unity. Such perturbations are strongly unstable. They grow more rapidly than any exponential with a growth time that becomes far less than t_{cool}.

As a numerical example, the activation temperature in a neutron star is of the order 10^8–10^9 K. Suppose we give the star a 10% thermal perturbation. Then the temperature increases with a growth time of the order of the cooling time at 10^7 K, but between 10^4 and 10^{40} times faster at 10^6 K.

As the temperature rises the frictional coupling between the pulsar angular velocity Ω and that of the superfluid ω will increase. The torque equations governing Ω and ω have been written in the two-component model by Baym *et al.*[1] (their equations (1) and (2)). The steady-state solution of these equations is $\dot{\omega}_0 = \dot{\Omega}_0$ and $(\omega - \Omega)_0 = I\tau_0|\dot{\Omega}_0|/I_c$. We will look at perturbations in the slowing-down rates $\dot{\Omega}$ and $\dot{\omega}$: write

$$\begin{aligned}\dot{\Omega} &= \dot{\Omega}_0[1 + \delta(t)] \\ \dot{\omega} &= \dot{\Omega}_0[1 + \gamma(t)]\end{aligned} \tag{10}$$

Combining equations (6) and (10) with the torque equations we obtain

$$\gamma = -(I_c/I_n)\delta \tag{11}$$

and the basic equation governing the fluctuations in the slowing-down rate of the pulsar (to first order)

$$\dot{\delta} = -\dot{\varepsilon}\frac{I_n}{I_c}\left(1 + \frac{T_a}{T_0}\right) - \frac{\delta}{\tau_0}\exp\left\{\frac{T_a}{T_0}\varepsilon\right\} \tag{12}$$

Equation (12) shows that in the absence of thermal perturbations the system is mechanically stable: with $\varepsilon = 0$ it reads $\dot{\delta} = -\delta/\tau_0$ which describes the classic spin-down problem of Baym *et al.* On the other hand, the mechanical response to the runaway heating described by equation (9) is, for small δ

$$\dot{\delta} = -\dot{\varepsilon}\left[\frac{I_n}{I_c}\left(1 + \frac{T_a}{T_0}\right)\right]. \tag{13}$$

Equation (13) shows that a thermal runaway (positive ε) is accompanied by a decrease in the slowing-down rate of the pulsar (negative δ). Because the factor in square brackets in equation (13) is greater than unity, the time scale for this decrease is even shorter than that for the thermal runaway.

Discussion

It is well known that, when born in a supernova explosion, a neutron star will be exceedingly hot: its temperature will greatly exceed the critical temperature $T_a/3$ separating stable from unstable configurations. It will therefore initially be stable against thermal perturbations. What is the subsequent thermal history of the star? I have previously[2] shown that the steady-state frictionally generated temperature of the star T_0 decreases as the pulsar ages. This means that the pulsar begins life in the configuration shown in Fig. 1*b*. The intersection point between the solid and dashed lines drifts towards lower temperatures as time passes; furthermore, as shown here, while this happens the slopes of these two lines become more nearly equal at their intersection point. When T_0 reaches $T_a/3$ the lines have become parallel at T_0. The star is now neutrally stable against thermal perturbations. When the temperature drops still further the situation switches to that sketched in Fig. 1*a*, and the star has become unstable.

Because the system is strongly unstable only for finite perturbations we must have some idea as to what could cause such initial perturbations. The smaller the necessary 'triggering' perturbation, however, the less seriously one need take this requirement. Equation (9) shows that a perturbation to the temperature greater than $\varepsilon_{\min} \simeq T_0/T_* \simeq T_0/(10^8\text{–}10^9)\text{K}$ will grow rapidly. For plausible stellar temperatures this can be quite small. Note also that in the binary X-ray pulsars accreting matter can easily supply the necessary perturbations. Furthermore, in the isolated pulsars a neutron starquake may release enough thermal energy to trigger the instability. As a numerical example, Baym and Pines[6] estimated that $\simeq 10^{41}$ erg can be released in a typical 'small' starquake. At a temperature of 10^6 K this yields a 100% change in the temperature for reasonable specific heats: the growth time for the subsequent runaway is of the order of exp{1,000} shorter than the cooling time.

What is the ultimate fate of the instability? As the temperature increases the pulsar tends to lock rotation with the more rapidly rotating superfluid. (The decrease in slowing-down rate described above is the first stage of this process.) There is no reason to believe that once the perturbation has become large this behaviour suddenly ends. In fact, it will end only when the two components have rigidly locked together. At this point the frictional generation of heat ceases and the star commences to cool. By how much has the pulsar sped up in this process? From considerations of angular momentum conservation it is easy to show that the fractional change in pulsar period will be

$$\frac{\Delta\Omega}{\Omega} = \frac{I_n}{I}\left(\frac{\omega_0 - \Omega_0}{\Omega_0}\right) \tag{14}$$

that is it is of the order of the initial fractional lag between superfluid and crust. Detailed studies[2] show that this lag can be quite large: the mechanism seems capable of producing large period jumps such as have been observed in the Vela pulsar. At any rate, the fact that a sufficiently cool pulsar can exhibit the instability demonstrates that its rotational state must be radically different from that of a hotter one.

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Massive deep-sea sulphide ore deposits discovered on the East Pacific Rise

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Massive ore-grade zinc, copper and iron sulphide deposits have been found at the axis of the East Pacific Rise. Although their presence on the deep ocean-floor had been predicted there was no supporting observational evidence. The East Pacific Rise deposits represent a modern analogue of Cyprus-type sulphide ores associated with ophiolitic rocks on land. They contain at least 29% zinc metal and 6% metallic copper. Their discovery will provide a new focus for deep-sea exploration, leading to new assessments of the concentration of metals in the upper layers of the oceanic crust.

THE area of the deposits of ore-grade zinc, copper and iron sulphide was explored and sampled in February–March 1978 by the manned diving saucer CYANA during the expedition CYAMEX[1]. The expedition, the only submersible diving programme that has so far been conducted on the East Pacific Rise (EPR), is part of the French–American–Mexican project RITA (Rivera–Tamayo), a 3-yr study devoted to detailed geological and geophysical investigations of the EPR crest. The ore deposits were sampled in water depths of close to 2,620 m at two neighbouring sites near 20° 54′ N 109° 03′ W, (refs 2–5) about 90 km north of the Rivera transform fault and 240 km south of the Tamayo transform fault (Fig. 1). Three dives of Cyana (CY 78–06, 08 and 12) crossed the two sampling sites, and we collected samples during two of these dives (CY 78–08 and 12). However, during all dives in the EPR axial zone, signs of hydrothermal activity were seen, including colonies of dead giant clams, fields of pillow lavas with pronounced colour-staining at the base of pillows, and coloured deposits on exposed scarp surfaces of normal faults and open fissures[1]. Coral-like growths, possibly of native sulphur, occur in other locations, including a sedimented fault-scarp about 1.0 km to the west of where the sulphide ores were sampled.

Sampling sites

The two sites where the sulphides were sampled lie on the lightly sedimented flanks of steep-sided structural depressions, about 20–30 m deep, 20–30 m wide, and about 600–700 m west of the

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*The authors are all members of the CYAMEX Scientific Team.

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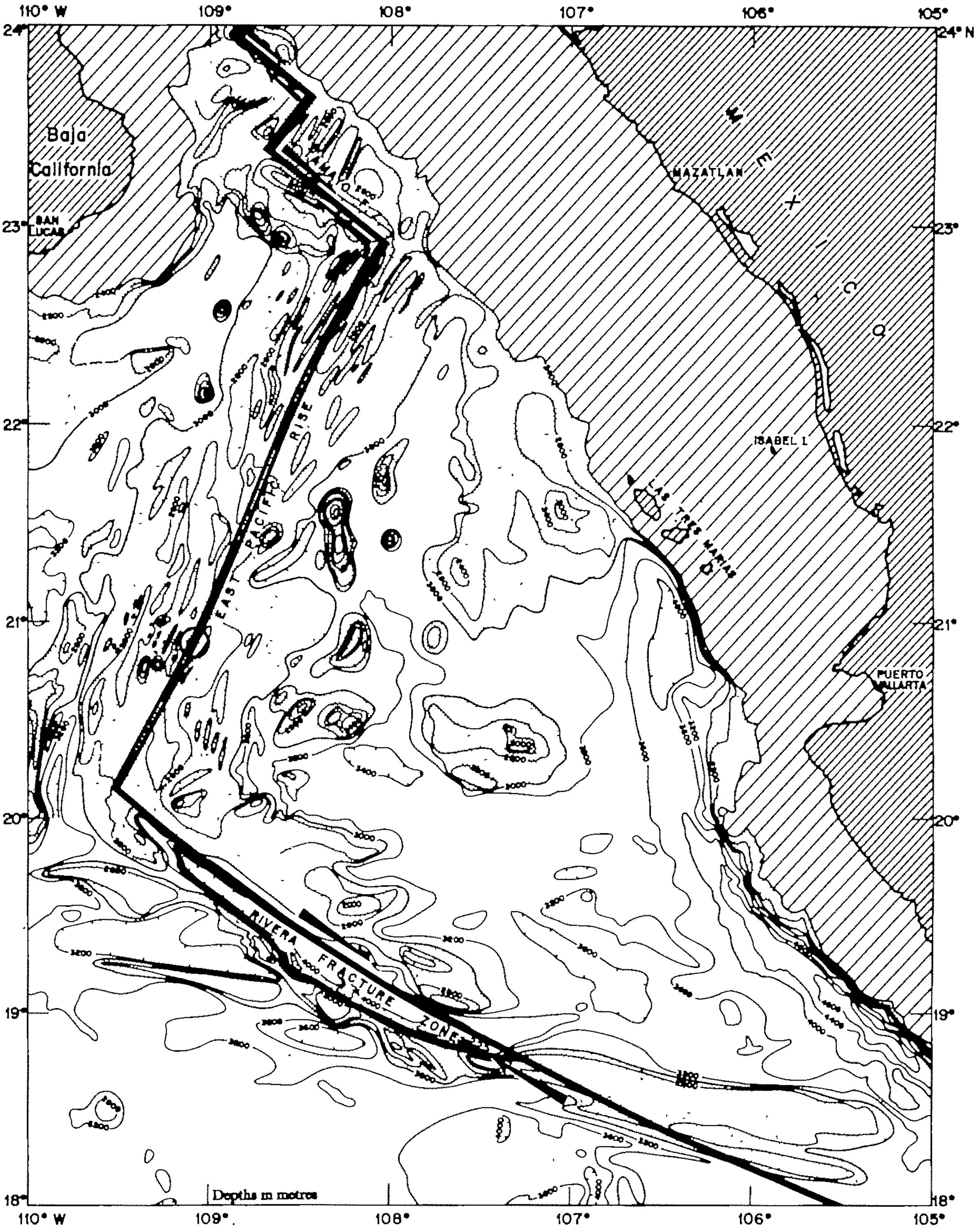


Fig. 1 Setting of the axial zone of the East Pacific Rise where the sulphide ore deposits were explored and sampled (circle).

axis of the 'extrusion zone' where the youngest lavas occur. Whereas the extrusion zone is marked by a 50 m-high sediment-free discontinuous ridge with no fissures or faults, the structural zone within which the sulphide deposits are located is a 1.5 km wide tectonically active band of fissured and/or faulted terrain, the fault pattern being typically of the small horst and graben type and with all the active structures running parallel to the local strike of the EPR.

The massive sulphides were sampled from various parts of tall (up to 10 m-high), extremely irregular columnar edifices about 5 m in diameter (Figs 2 and 3). At one site, explored in relative details during dive CY 78–08 (Fig. 2), the vertical edifices are 4–5 m apart and are aligned along a direction of 025° over a distance of at least 50 m, in gullies cutting through the 045°-trending, westwards-facing scarp of a structural depression (graben). The second site, explored during dive CY 78–12, is about 200–300 m away and lies on the eastwards-facing flank of a possibly older graben. Here, there is more sediment cover, there are yellow-coloured coral-like growths and the edifices seem to be more indurated. The true spatial relationship of the two sampled sites is not established because of navigational difficulties during dive CY 78–12.

The along-strike extent of the sulphide mineralisation is not known. However, during dive CY 78–08 the graben was followed for about 100 m to the SSW (200°) where it became a 2–3 m wide open fissure with truncated pillows entirely stained by ochre and red-brown deposits exposed on the scarp. The eastern lip of the fissure has small (about 0.5 m high), sponge-like porous edifices and coral-like, bright yellow growths on sediment between pillows. Further south the fissure narrows and is bare of any hydrothermal deposits.

In general, the vertical edifices are built on a pillow lava terrain covered with a light dusting of sediment, the colour of which suggests some contribution of weathering products from the deterioration of the columnar edifices themselves. The edifices are variegated, the dominant colours being ochre, red, yellow, brown, white and black. They are porous and their entire mass is composed of a labyrinth of small channels or tubes separated by thin walls. Their internal structure can be compared to that of a sponge or a mass of clinker or brains. The framework of the edifices is made up of both amorphous, white translucent silica (which has been seen bare in one case) and the sulphides. The presence of open vents on top of the edifices demonstrates that they are constructional features built directly on the ocean crust. For this reason, we described them as hornitos when first observed. The tubes, conduits and channels pervading the mass of the edifices presumably represent the passages through which hot, metal-laden solutions circulated on their exit from the sea floor.

Three other modes of mineralisation occur besides the spectacular vertical edifices: (1) flattish incrustations coating the moderately steep slopes with a rugose, heterogeneous surface texture made up of the same material as the tall edifices; (2) bright yellow or orange, 10–20 cm wide conelets built on sediment and with small holes at their tops and which lie around the large edifices; (3) yellow and red-brown travertine-like flows draping the near-vertical scarps of the graben that contains the row of vertical edifices. No unequivocal bottom-water temperature anomaly was observed during the exploration of any of the mineralised structures, suggesting completed or quiescent hydrothermal activity.

The material collected from each of the two sampling sites was separated on board ship according to colour and shape, giving a total of 14 samples (three from the site sampled during dive 8 and 11 from that sampled during dive 12). Some of the 14 samples thus designated comprise several subsamples or collections of fragments. A total of 215 subsamples and 11 boxes of small fragments were separated in the laboratory. The samples are very heterogeneous and complex, and no attempt is made at this stage to give a full description of them. We describe here only the gross mineralogy and give an indication of the chemistry of a few important subsamples.

Mineralogy and chemistry

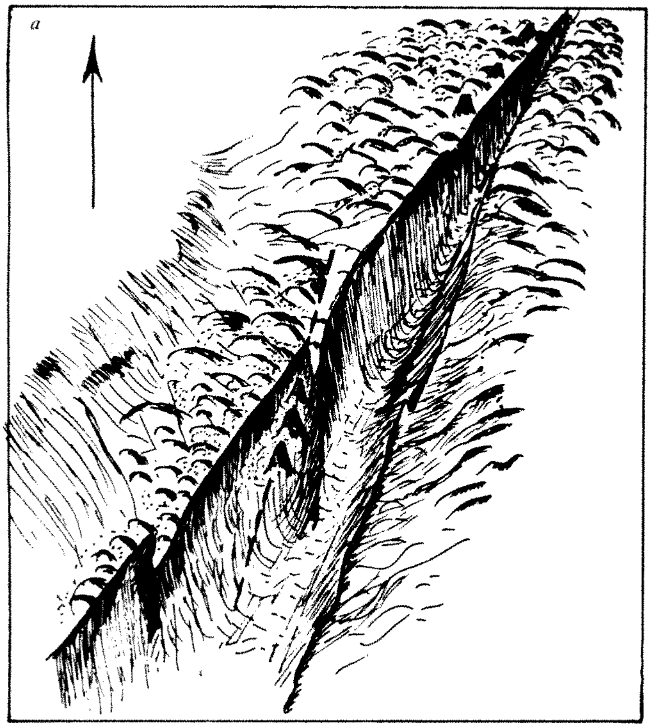
The material consists of friable and porous compounds that are easily fragmented; thus some subsamples are as large as 7 cm in diameter but many are smaller, and some consist of debris with diameters of one to a few mms, or are essentially powder. The subsamples are variegated: yellow to ochre-coloured, grey and blue metallic hues, and grey, bronze red and white reflections. The ochre-coloured material ranges from pale yellowish orange (10 YR 8/6) and moderate yellowish brown (10 YR 5/4) to moderate brown (5 YR 4/4) and from blackish red (5 Y 2/2) to moderate red (5 R 4/6). Most subsamples show well developed cylindrical tubes that display a concentric zonation of medium grey (N 5) layers on the outside to dark gray (N 3) and white (N 4) layers inside. Other tubes are composed of the lighter-coloured moderately brown material (5 YR 4/4). The inner walls of some of the tubes are lined with shiny, silvery deposits that are in part microcrystalline, whereas others are covered only by reddish deposits. Some tubes are lined with black deposits inside and coated with yellow and ochre-coloured deposits outside. Other tubes are filled or partially filled by black material. The tubes are variable in size (0.1–1.5 cm in diameter and up to 8 cm in length) and are irregularly scattered throughout the subsamples. Some tubes are closely spaced in a parallel fashion.

Five pieces from four samples were selected for macroscopic examination and preliminary mineralogical and chemical analysis. Four of the pieces (Cyp 78-12-38A-2, 38A-1-a, 40A-a and 40B) are relatively dense and consist mainly of sulphides. The fifth (Cyp 78-08-14A-1) is oxidised and less dense than the others. Subsample Cyp 78-12-40 B is elliptical in shape; the convex side is coated with dark, heavy sulphide aggregates and

Table 1 Mineralogy and chemistry of hydrothermal samples from 21° N on the East Pacific Rise (major and minor element content determined by X-ray fluorescence and atomic absorption spectrometry)

Subsample or specimen	Approx size (cm)	Weight (g)	Microscopy: Minerals	Microscopy: Morphological features	X-ray diffraction: Minerals	X-ray diffraction: Peaks (Å) d(Å)	Metal content (wt%): Fe	Metal content (wt%): Cu	Metal content (wt%): Zn	Other identified elements through XRF
CYP 78 12-40 B	6.5×4×4	90.7	Sphalerite	Tabular, polysynthetic twins, octahedron	Sphalerite Pyrite Chalcopyrite	3.12; 1.91 2.70, 2.42, 1.63 3.05	9	2.9	23	Co*·Pb*·Ag* Cd* Mn*·Cl* S† Ca*·K*
			Pyrite	Cubes with striation						
CYP 78 12-40 A-a	4×4×1	64.7	Sphalerite	Octahedron, collomorph, banded	Sphalerite Pyrite Chalcopyrite (?)	3.13; 1.91 2.71, 2.42; 1.63 3.06 (?)	6.3	0.2	28.7	Co*·Pb*·Ag* Cd*·Mn*·Cl* K*·Si†·S†
			Amorphous silica	Flaky						
CYP 78 12-38 A-2	4×4×2	26.5	Pyrite or chalcopyrite or sphalerite	Globular and collomorph, tetrahedron	Pyrite Chalcopyrite	3.13, 2.71, 1.63 3.06	29.6‡	6‡	non analysed	Co*·Cd*·Pb* Mn* Zn* Ca* K*·Cl*·S†
CYP 78 12-38 A-1-a	4×2×2	74.3	Pyrite	Cubes in lamellae, octahedron with striations	Pyrite Marcasite Chalcopyrite (?)	2.71; 2.42; 1.63 3.44; 2.71, 1.76 (3.04)?	14.9	2.2	0.0	Mn*·Cd*·Co* Pb* Ag*·Zn* K* S†
			Pyrite or chalcopyrite or sphalerite	Globular and collomorph						
CYP 78 08-14 A-1	6×5×1.5	19	Iron oxides	Globular aggregates	Amorphous	Broad	42.7§	0.0	0.6	Mn*·Pb*·Cu* Ca†·Cd*·K* S†

*Few tens to few thousands p.p.m. †Few % to few tens of %. ‡ Analysed by XRF. § Red part of subsample CYP 78-08-14A-1.



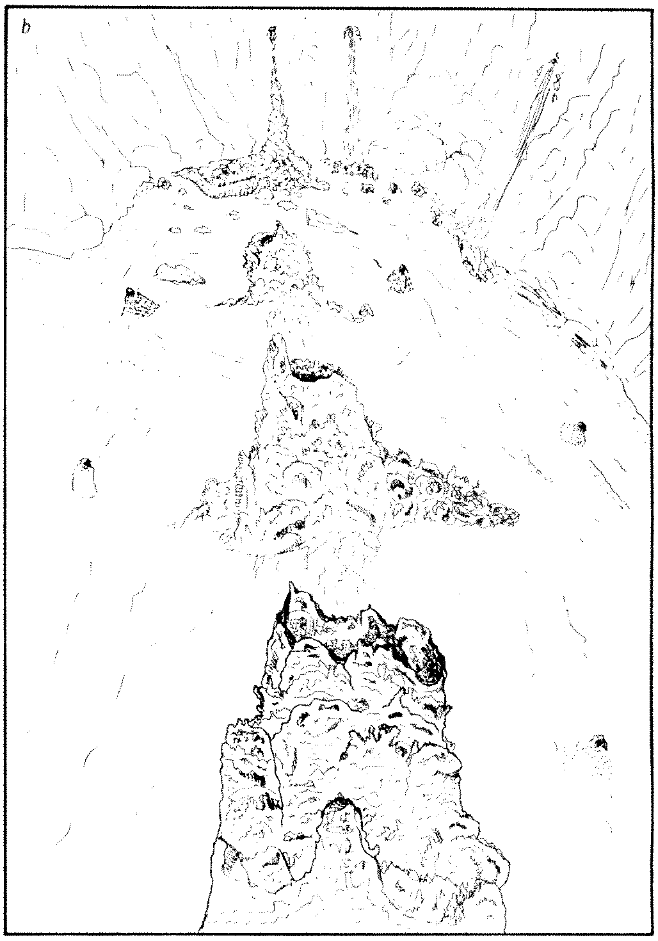


Fig. 2 *a*, Overall sketch of the 225° trending structural depression with a small gully or re-entrant trending 205° that crosses the east wall of the graben (dive CY 78-08). The width of the graben is about 20–30 m and narrows towards the SSW. The arrow points towards the south. *b*, Artist's view from CYANA's porthole of the site of sulphide ore edifices on the eastern flank of a structural depression (dive CY 78-08). The view is towards the south. The hornito-like edifices are aligned along 205 in a gully cutting across the graben. The spacing between 'hornitoes' is about 5 m. The small conelets associated with the vertical edifices are shown as well as the flows of hydrothermal material on the vertical scarp in the cul-de-sac of the small gully. All the sulphide material was sampled from the porous edifices.

there are patches of greenish-yellow dust with a strong odour of sulphur. The concave surface of the specimen is coated by moderate yellowish brown and brownish ochre material. Small empty tubes (0.1–1 cm in diameter) are also seen. Specimen Cyp 78-12-40A-a is a nearly black material with circular tubes, one of which has its internal wall coated by a brass-yellow lamella. Concentric zonation of dark (outside) and white (inside) lamellae is exhibited by another adjacent tube. Specimen Cyp 78-12-38A-2 is dark grey with remnants of tubes and patches of yellowish-green, green and purple brown material similar to the other dark sulphide aggregates. Specimen Cyp 78-12-38A-1-a is a massive aggregate of black sulphide-rich material which is extremely friable and porous and gives off a strong odour of sulphur. It contains specks of yellowish-green material which could represent a sulphur oxide compound. Subsample Cyp 78-08-14A-1 is a moderate brown to blackish red to ochre-coloured powdery material with abundant relicts of tubes, some of which are open and continuous across the specimen, while others are filled by a yellowish brown material. Sulphide material coats some of the walls of the empty tubes.

The mineralogy of the material was determined by X-ray diffraction after microscopic examination in reflected and transmitted light (Table 1). The ochre-coloured material consists mainly of amorphous iron-oxide. The oxides are reddish and yellow and are found in the interstices of the sulphide grains, resulting primarily from secondary alteration of the sulphides.

The sulphide phases consist mainly of sphalerite and pyrite with minor chalcopyrite and marcasite. The sulphides are represented by agglomerates of intimately associated, well-crystallised phases and amorphous material. The most common form encountered in the samples is an octahedron. Tetrahedra characteristic of sphalerite, cubes of pyrite, bipyramids, sphenoids characteristic of chalcopyrite, and tabular crystals with polysynthetic twins (probably of sphalerite) are also observed. Globular and reniform associations, probably consisting of chalcopyrite and pyrite, with anisotropic clay-size material are commonly associated with the well-crystallised sulphides.

The chemistry of the five specimens was determined qualitatively by X-ray fluorescence (XRF) spectrometry (dispersive) and quantitatively by atomic absorption spectrometry (Table 1). The XRF analyses show the presence of abundant zinc, iron, copper and sulphur and of lesser quantities of several other elements including cobalt, lead, silver and cadmium. The material used for atomic absorption analyses (100 mg to 1 g) should be reasonably representative of the subsamples which weigh from 19 to 91 g. The analyses show two principal modes: one zinc-rich (23–28.7%) and the other iron-rich (19.9–42.7%). Three specimens are copper-rich (2.2–6%) and we note the absence of manganese even as a minor element.

The subsamples can be divided into two major types according to their macroscopic features, mineralogy and chemistry: (1) the dark-coloured and porous, zinc, iron and copper sulphides in the form of sphalerite + marcasite, pyrite and chalcopyrite, with a skeleton made up of a white unidentified material; and (2) the fragile, pulverulent ochre-coloured material made up of amorphous iron oxides. The widespread occurrence of pure sulphur on many of the subsamples suggests that the small yellow conelets and bright yellow crystal-like growths around them, which are associated with the larger variegated edifices on the sea floor, might represent massive concentrations of native sulphur.

The occurrence of sulphide mineralisations on the sea floor in an active tectonic region directly associated with an accreting plate margin can best be explained by a model in which the deposits formed within the discharge zone of a hydrothermal circulation system involving seawater as the convecting fluid[6–12]. A similar explanation has been put forward for the genesis of ophiolitic sulphide ore deposits on land, which share many common characteristics with the EPR deposits[6,9–17]. A typical example of the ophiolitic sulphide ores is found in Cyprus[6,10,12,13–15,17–20].

The Cyprus deposits occupy basin-like depressions in basalts and occur as irregular, elongate lenticles or lens-shaped bodies of massive sulphide ores (pyrite + chalcopyrite ± sphalerite ± marcasite with minor galena, pyrrhotite, gold and silver). Like the EPR deposits, which are also restricted to structural lows, they accumulated on the sea floor before the first sediments were deposited and in some deposits the ore is overlain by pillow lavas and sediments. An interval of 10^5 years has been tentatively proposed for the formation of the Cyprus ores[12]. The EPR deposits are adjacent to the main axial extrusion zone but there is direct evidence from the dives that lava can also be emplaced within the horst and graben tectonic zone so that burial of the deposits by off-axis volcanism is possible. We estimate the age of the EPR deposits to be $<1–2\times10^4$ years using as a guide the fact that they occur on crust whose age is theoretically $1–2\times10^4$ years, based on spreading rate.

The Cyprus deposits can show alteration near the top to a geothitic material or to pyrite-bearing chemical sediments (ochres) enriched in silica and hydrated iron-oxides. The ochreous components of the EPR deposits are probably gossans and represent products of submarine weathering of the sulphide ores. The Cyprus deposits display prominent metal zoning with zinc-rich bodies lying deep in the Lower Pillow Lavas and larger copper-rich bodies at shallower levels in, or at the top of, the Upper Pillow Lavas. We have no evidence yet of whether or not there is metal zoning within the metre-scale sulphide cones of the East Pacific Rise. There are indications of some zoning at the scale of the samples. The sulphides on Cyprus exhibit primary breccia textures in the upper part and are massive in the lower part. Ore textures similar to the sponge-like texture of the EPR deposits have not been described in Cyprus or other Cyprus-type sites but the Cyprus ores are said to be very porous. The fragile tubular and heterogeneous texture of the EPR sulphides may not be preserved in the geological record or possibly they represent the equivalent of the upper breccias in Cyprus ores. Finally, the Cyprus ore deposits are separated by a mean distance of about 2.5 km in a direction perpendicular to the strike of the sheeted dikes and thus to the strike of the fossil spreading centre. The hydrothermal sulphur/sulphide sites of the EPR are separated by about 1 km in a direction transverse to the EPR axis.

Conclusions

Despite the differences in detail and in scale between the East Pacific Rise deposits and the Cyprus ores, there are enough general similarities in the type and style of metal concentration to regard the EPR deposits as a modern analogue of the zinc-rich cupriferous massive sulphide deposits associated with ophiolite complexes. The EPR ore deposits, which have their primary features still preserved and which have been emplaced in a well defined geological setting directly associated with plate accretion, should shed a new light on the characteristics of, and genetic models for ophiolitic sulphide ore deposits such as found in Cyprus[6,10,12,13–15,17–20], Newfoundland[16], California[10], Turkey[10], and Italy[9]. The EPR deposits further demonstrate that modern ocean floor sulphide deposits are not restricted to the initial stages of ocean rifting, as suggested by the Red Sea deposits.

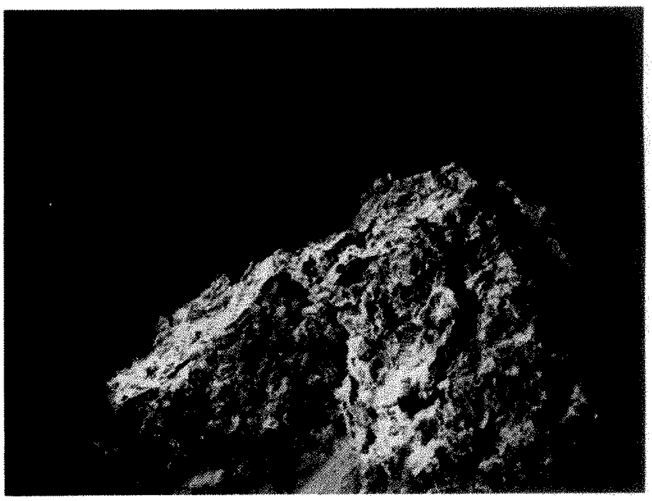


Fig. 3 Photograph of a variegated hornito-like edifice taken during dive CY 78-06. The diameter of the edifice is ~2 m. A second edifice can be seen in the background.

The metal-rich brines and muds that are found in the axial region of the Red Sea[21], or beneath the Salton Sea Trough[22] (a northwards extension of the Gulf of California) show iron-rich layers overlying a sulphide horizon with sphalerite and less abundant chalcopyrite, pyrite and marcasite. In contrast with the sulphide-rich, Mn-deficient EPR deposits, the other metalliferous deposits encountered in Mid-Ocean Ridge settings are essentially Fe–Mn concretions, Fe–Si rich clay material, and occasional pure MnO_2 deposits[23–26].

Mid-Ocean Ridge sulphides and ferromanganese deposits attributed to a hydrothermal origin are all chemical precipitates which form at the basalt–seawater interface when hot seawater solution, laden with Fe, Mn and other metals stripped at 'high temperature' (see refs 8, 10, 12) from the ocean crust, debouches onto the ocean floor. Sulphides, such as the EPR deposits, can be preserved either if the bottom waters are anoxic or if the sulphide deposits become rapidly buried under sediments or lavas. The ochres, such as those of the East Pacific Rise, result from the oxidation of the iron sulphide. The Fe–Mn deposits (umbers) encountered in the FAMOUS area, in the Galapagos and in the Gulf of Aden may be the product of a weaker flow of the convecting fluid although many other factors (such as temperature, redox conditions, and fugacity) may be critical. Sulphides in such settings would be deposited subsurface. Indeed, this is suggested in the FAMOUS area by the presence of pyrite in the deposits near the vents[24]. It is not clear whether the flow rate of ocean floor convective systems depends primarily on the spreading rate but it seems from the contrast between the RITA and Galapagos areas and the FAMOUS area that hydrothermal activity is more prevalent in higher spreading-rate environments.

It will be a test of ingenuity to assess the surface distribution of metal-rich deposits on the Mid-Ocean Ridge, let alone their distribution at depth. Furthermore, there is the problem that unusual circumstances might be required for their preservation. However, the discovery in the RITA area shows that metal deposition is not restricted to young oceans and that the axial zones of medium-fast (and perhaps fast) ridges are not only factories of oceanic crust and lithosphere but also plants for the concentration of heavy metals through leaching of the oceanic crust in zones of intense hydrothermal activity and deposition on the basaltic floor. Indeed, the EPR deposits throw a fresh light on the whole question of the concentration of metal deposits of the oceanic crust.

In the future one might envision that submarine metalliferous ore deposits are distributed in quasi-continuous, linear strips several thousand kilometres in length along the axis of the fast-spreading East Pacific Rise and south-east branch of the Mid-Indian Ocean Ridge. Certainly we will have to re-examine our estimates of the mineral resources which lie within reach on top of the bare crust of the Mid-Ocean Ridge. Slow ridges seem so far to yield only Fe and Mn-rich deposits in fracture-zone terrain; thus, the sites of priority interest for further sulphide ore exploration are the fast as well as super-fast ridges.

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Friction and seismic attenuation in rocks

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Precise experimental results, combined with theoretical predictions, indicate that seismic energy loss caused by grain boundary friction is important only at low confining pressure and at strains greater than about 10^{-6}. *Since these conditions are generally not encountered in seismology, frictional attenuation is not important* in situ. *Other mechanisms such as fluid flow must dominate seismic attenuation in the upper crust.*

FAR more information about the constitution of the Earth is obtained from seismic studies than from all other geophysical methods combined. Of the two main aspects of wave propagation—velocity and attenuation—knowledge of velocities has provided most of our information about the Earth. Attenuation data is of much more limited use, partly because it is difficult to obtain, but mainly because it is difficult to interpret in terms of rock properties. This is due primarily to our lack of understanding of the physical processes involved in the attenuation of seismic waves. (Here we are not considering apparent attenuation caused by geometrical spreading, partial reflections and so on, but are only interested in those processes that convert seismic energy into heat.)

Many different techniques have been used to study the attenuation of acoustic waves propagating through rock. Although much data has been collected, no well-defined mechanism of energy loss has yet been firmly established on both experimental and theoretical grounds. One of the most intuitively appealing and widely discussed mechanisms proposed for seismic energy loss is based on simple Coulomb friction. In this mechanism the passing wave causes sliding at grain boundaries or across crack faces, thereby doing work against friction and converting seismic energy into heat. Since frictional heating is rate independent, it seems to account for the reported observation that the specific dissipation function[1], Q, is nearly independent of frequency. Friction also seems to explain why the introduction of cracks into a crystalline solid increases attenuation. The most severe criticism of a frictional mechanism is, however, that the mechanism is inherently nonlinear. This means that the velocity and attenuation of a sinusoidal wave will depend on how it is combined with other waves. It has been found that at large strain amplitudes ($>10^{-6}$) nonlinear effects such as cusped hysteresis loops[3,4] and amplitude-dependent velocity and attenuation[3,5,6] can be observed, but these effects disappear at strains more typical of seismic waves.

Using both theoretical arguments and experimental results, several authors[2,7–9] have expressed doubts about the validity of a frictional attenuation mechanism. For instance, Savage[7] has suggested that frictional effects should be important only at large strains where displacements across crack faces are sufficiently large for the concept of macroscopic friction to be valid. Despite these criticisms, frictional losses are often used to interpret experimental observations[10–12]. To clarify the role of friction in seismic wave attenuation, we carefully investigated the dependence of velocity and Q on strain amplitude. We were able to identify the range of conditions for which frictional losses may be significant and our results show that the frictional mechanism is generally not important for seismic waves in the Earth.

Theory of frictional losses

Two types of frictional attenuation models have been proposed. Walsh[13] developed a model based on thin elliptical cracks in which frictional sliding occurs between crack faces. Models based on spheres in contact were developed by Mindlin and Deresiewicz[14] and by White[15]. Mavko[16] has reviewed these theories and extended the contact model to more general geometries. The most significant feature of Mavko's model (which it shares with that of Mindlin and Deresiewicz) is that it predicts Q^{-1} to be proportional to strain amplitude. It has been

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found experimentally that frictional behaviour is often described by an equation of the form[17]

$$\sigma_f = S + \gamma\sigma_n$$

where σ_f is the frictional force, S is the cohesive shear strength, γ is the coefficient of friction and σ_n is the normal stress across the sliding surfaces. The contact models predict that attenuation will decrease as either γ or S increases. Attenuation will also decrease as σ_n increases and the sliding interfaces are effectively locked together.

In addition to the effects on attenuation, all frictional models predict that stress–strain loops will have discontinuities in slope at maximum strain. This is a static hysteresis effect. As stress reverses direction there is a simultaneous reversal of elastic strain and a delayed reversal of sliding strain.

Table 1 Sample descriptions

Sample	Dimensions (cm)	V_E/f_E (km s^{-1} Hz^{-1})	Porosity
Massilon sandstone	100×2.0×1.9	2.000	22%
Berea sandstone	101.6×2.54× 2.54	2.032	20%
Sierra White granite	101.6×2.54× 2.54	2.032	1%
Vycor 7930 glass	91.4×1.5 diam.	1.828	28%
Lucite (plastic)	98.7×1.51 diam.	1.974	0%
Aluminium	100×2.22×2.22	2.000	0%

Vycor 7930 porous glass is made by Corning Glass Works; the average pore size is 4 nm.

Past observations of frictional losses

Several of the predicted effects of frictional attenuation models have been observed. Energy loss has been found to increase with strain amplitude at large strains in rock[3] as well as between sliding glass and metal surfaces[19,20]. It has been shown that at low strains ($<10^{-6}$) attenuation is nearly constant[3] and that variation with strain amplitude occurs only at larger strain. Some attenuation in metals at large strain is associated with dislocation mechanisms, but this does not explain all of the observations.

Discontinuities in the slope of stress–strain loops have been observed in large strain quasi-static experiments[3,4]. These have been described as cusped loops. It has recently been shown[21] that as strain amplitude decreases, the cusped loops become elliptical at strains approaching 10^{-6}. It is interesting that two of the effects predicted by frictional attenuation models, amplitude-dependent Q and cusped hysteresis loops, both seem to disappear below the same strain amplitude.

It is also commonly observed that increasing confining pressure causes attenuation to decrease[22,23]. This is predicted by frictional models, but it is also predicted by many other possible mechanisms of energy loss and so by itself does not provide evidence for frictional loss.

New observations of frictional losses

We are using the well established bar resonance technique[22,23] to measure both attenuation and velocity in long thin bars of rock. (Experimental details will be presented elsewhere.) An important feature of our experiment is that pore pressure is controlled independently of confining pressure. This has not previously been achieved in a resonance experiment. The length of the samples (~1 m) puts the fundamental resonance frequencies at roughly 500–1,500 Hz. Sample dimensions are given in Table 1. Resonance frequencies are measured to 1 part in 10^3 and converted to velocities using the relation $v = 2Lf$ where L is the length of the bar. Geometric corrections given by Spinner and Tefft[24] are negligible for our samples in extensional resonance. Strain amplitudes are measured with crystal phonograph pick-ups calibrated against semiconductor strain gauges.

Table 2 Tests for nonlinear behaviour in various materials (negligible changes of Q_E and V_E are found for materials where frictional mechanisms are inoperative)

Material	Strain amplitude	Q_E	Velocity (m s^{-1})
Lucite	1.43×10^{-6}	23.4±0.3	2,108
	3.04×10^{-8}	23.2±0.4	2,108
Vycor	2.84×10^{-6}	437±2	3,345
	1.58×10^{-8}	435±4	3,345
Aluminium	2.41×10^{-6}	34,800±300	5,102
	2.46×10^{-8}	35,200±400	5,102
Granite	1.44×10^{-6}	185±1	3,629
	4.15×10^{-8}	204±1	3,637
Massilon sandstone	2.10×10^{-6}	107±1	1,820
	1.55×10^{-8}	138±2	1,834
Berea sandstone	2.10×10^{-6}	103±3	1,937
	2.30×10^{-8}	140±2	1,955

Attenuation measurements can be made either by plotting resonance peaks ($Q = f/\Delta f$) or by measuring the time constant of resonance decays ($\tau = Q/\pi f$). Before using either technique, resonance peak shapes were carefully observed to insure the purity of the resonances. For convenience, all attenuation data presented in this article was taken with the resonance decay technique (except as noted in Fig. 1). The precision of the attenuation measurements is 1% and the estimated absolute accuracy is 5–10%. Both torsional (shear modulus) and extensional (Young's modulus) resonance modes show similar nonlinear behaviour at large strains. Only extensional data (taken at the fundamental resonance) will be presented here.

Data for dry Massilon sandstone as a function of resonant strain amplitude are shown in Fig. 1. At strains below 5×10^{-7} both attenuation (Q^{-1}) and velocity are nearly independent of strain amplitude, whereas at relatively large strains ($>10^{-6}$) there is a clear increase in attenuation and decrease in velocity. Of the two measured quantities, Q^{-1} is more sensitive to strain

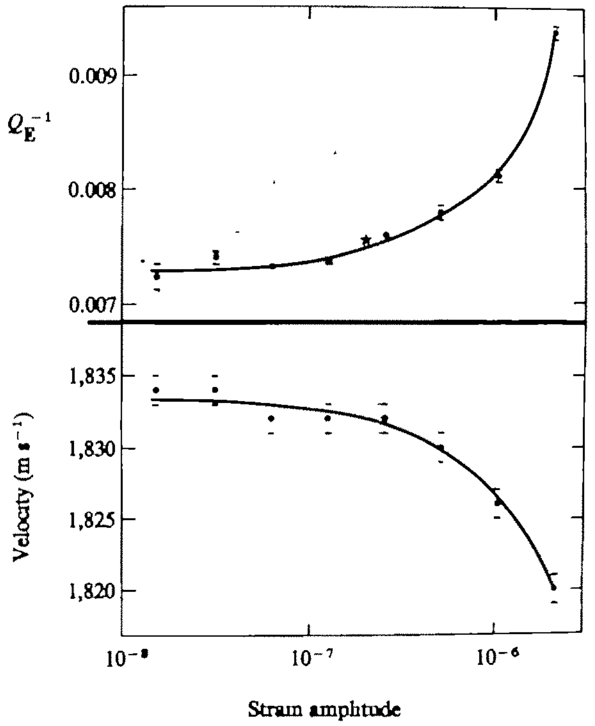

Fig. 1 Variation of attenuation (Q^{-1}) and velocity with strain amplitude for dry Massilon sandstone in extensional resonance. ★, The value found from the resonance peak half-width using the relation $Q^{-1} = \Delta f/f$.

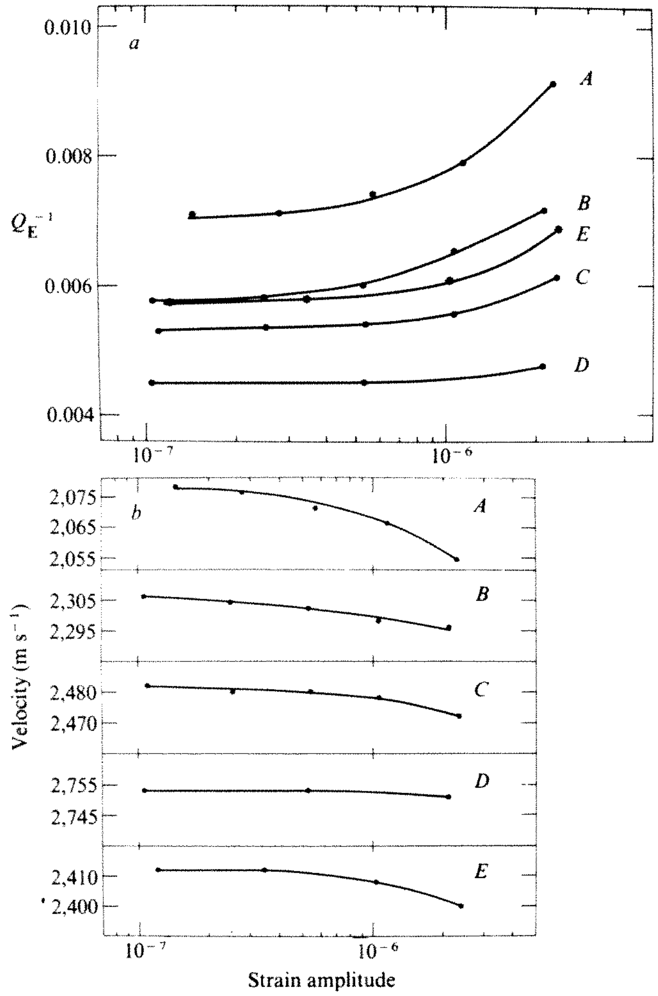

Fig. 2 *a*, Attenuation in dry Berea sandstone as a function of strain amplitude, confining pressure (P_c) and pore pressure (P_p). Helium was used as the pressure medium. (*A*) $P_c = 10$ bar; (*B*) $P_c = 20$ bar; (*C*) $P_c = 30$ bar; (*D*) $P_c = 50$ bar; (*E*) $P_c = 50$ bar, $P_p = 30$ bar. *b*, Extensional velocity of Berea sandstone. Data correspond to the attenuation data shown in (*a*).

amplitude than is velocity, varying by 19% and 0.7%, respectively. The similarity of the curve shapes suggests an underlying relationship between the amplitude dependence of Q^{-1} and velocity.

We have further investigated these nonlinear effects by varying effective stress—the difference between confining pressure and pore pressure. Figure 2 shows data for a jacketed sample of Berea sandstone, with helium used for both confining pressure and pore pressure. As confining pressure is increased from 10 to 50 bar (curves *A*–*D*), the variation of both Q^{-1} and velocity with strain amplitude gradually decreases. At a constant confining pressure of 50 bar (curves *D* and *E*) increasing pore pressure restores the dependence on strain amplitude. In Fig. 2*a* curves *B* and *E* show that Q^{-1} is almost a unique function of effective stress at a given strain amplitude.

The effects of changing water content on Q^{-1} in Berea sandstone are shown in Fig. 3. The data for partially saturated rock at very low saturation were obtained at ambient room conditions. Data for 'dry' rock were obtained after drying the sample in a vacuum oven, and the data for saturated rock after saturating the sample with distilled water. The most noticeable feature is that the addition of water greatly increases the attenuation. This effect, which is associated with a fluid flow mechanism, will be discussed more thoroughly elsewhere[29]. Of greater interest here is that the addition of a small amount of water greatly increases the variation of Q^{-1} with strain amplitude, whereas continued addition of water causes a smaller additional variation in Q^{-1}. This is indicated by the brackets in Fig. 3 which show the difference between Q^{-1} at a strain of 1.7×10^{-6} and the constant value of Q^{-1} at low strains.

To find out which structural features of a material are related to these nonlinear effects, we experimented with materials having physical compositions different from sandstone. Table 2 shows data for various materials measured at both large and small strain amplitudes. Lucite and aluminium show no evidence of any change in velocity or attenuation with strain (to within experimental uncertainty) although their respective Q values span more than three orders of magnitude and their internal structures are quite different. (The values of Q for aluminium are probably underestimates because they are at the limits of sensitivity of our system.) There is also no variation in the values for dry Vycor glass. Under the scanning electron microscope Vycor appears to be composed mainly of glass beads ~50 nm diameter. The contacts were beyond the resolution of the SEM used, (86,000×), but presumably the particles are welded together since they formed simultaneously from the melt. The flat cracks and partially cemented grain boundaries commonly found in rocks are probably scarce in Vycor. Table 2 also includes data for a room dry granite sample which shows the same nonlinear effects found in sandstone.

Discussion

The data we have presented, together with the existing data we have discussed, provide strong support for the existence of a frictional attenuation mechanism in rock at large strain amplitudes and low effective stresses. Every predicted feature of the contact frictional loss mechanism has been observed experimentally. At large strains ($>10^{-6}$) Q^{-1} increases with increasing strain amplitude and the amount of the variation in Q^{-1} is strongly influenced by effective stress. A stress of 50 bar is enough to virtually eliminate the strain dependence at the strains and in the rocks which we have used.

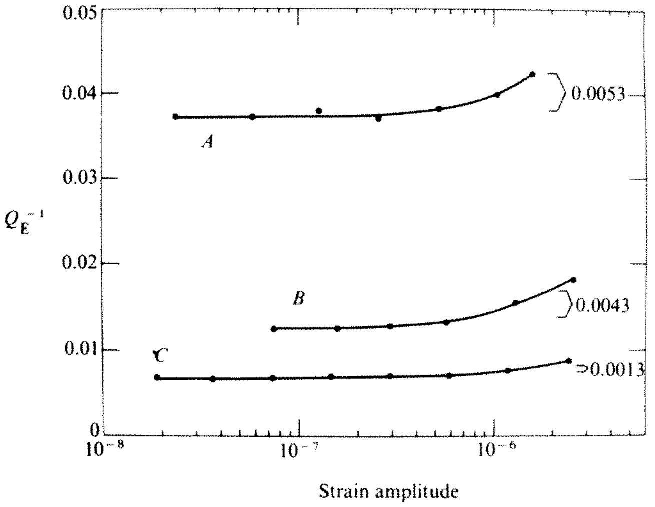

Fig. 3 Attenuation in Berea sandstone at various degrees of saturation as a function of strain amplitude. Brackets indicate frictional component of energy loss. *A*, saturated; *B*, partially saturated; *C*, dry.

The effect of water on the amplitude dependence of Q^{-1} is probably caused by water changing the frictional properties of the sliding interfaces. Figure 3 provides evidence for this. Adding a small amount of water to the sample presumably wets most of the internal surfaces, causing a slight increase in the low strain, linear attenuation (a fluid flow mechanism) and a large increase in the nonlinear, frictional component of attenuation. The addition of more water fills the remaining pore space, thus enhancing the flow losses but only slightly increasing the frictional energy loss.

The observations of cusped stress–strain loops[3,4] at strains greater than 10^{-6} also indicate that frictional sliding is occurring. It is significant that all of the evidence for a frictional attenuation mechanism disappears below strains of the order of 10^{-6}. The cusped loops become elliptical and Q^{-1} becomes independent of strain amplitude. The implication is that at low strains attenuation is caused only by linear loss mechanisms. At larger strains, nonlinear frictional losses become observable. This leads to the question of why a strain of 10^{-6} or larger is needed before frictional attenuation is observed. We can assume that displacements across crack surfaces must be at least comparable with inter-atomic spacings ($\sim 10^{-8}$ cm) for friction to be present. A shear strain ε will produce a displacement d across the sides of a crack of length L given approximately by $d = \varepsilon L$. If $d = 10^{-8}$ cm and $\varepsilon = 10^{-6}$, then we find that $L = 10^{-2}$ cm. A crack length of 10^{-2} cm may well be a rough upper bound to the majority of crack lengths found in rock. Hadley[25] reported that in Westerly granite only 7% of the observed cracks had lengths greater than 10^{-2} cm and none were longer than 3×10^{-2} cm. Therefore, at strains below 10^{-6}, sliding displacements between crack faces are so small in the majority of cracks that friction does not adequately describe the interaction. As strain increases above 10^{-6}, frictional sliding begins to occur at more and more cracks and attenuation increases.

After eliminating friction as a loss mechanism at low strains, we still have to account for the observed energy loss at low strain amplitudes in 'dry' rock. We must also explain the change in attenuation that occurs as cracks are closed under confining pressure[3,22,23] or created through thermal cracking[3] or dilatancy[11]. Unfortunately, we do not know yet what mechanism is responsible for these losses. The data collected by Tittmann *et al.*[9] showing the strong effect that trace amounts of volatiles have on attenuation probably has a direct bearing on this mechanism. Another possibility is some form of grain boundary relaxation[26,27] or a dislocation mechanism similar to those discussed by Mason[8,28]. Whatever the cause may be, it is reasonably certain that the mechanism does not involve any macroscopic concept of friction.

Conclusion

On both experimental and theoretical grounds it seems clear that frictional energy losses occur in rock at room pressure only at strains greater than approximately 10^{-6}. This is far greater than strain amplitudes generally encountered in seismology except in regions near seismic sources. Even there the effect of confining pressure will virtually eliminate frictional energy losses in the Earth. Consequently, frictional losses are not important in seismology.

The mechanism by which cracks influence attenuation at low strains in 'dry' rock needs to be re-examined. The concept of frictional energy loss is so firmly established (on intuitive grounds) that the term 'internal friction' is often interpreted literally to mean a frictional energy loss. Though the choice of words is unfortunate, internal friction is simply a general term for energy absorption and does not imply any particular mechanism. It must be kept in mind that actual frictional energy loss is a large-strain, low-pressure phenomenon that is often observed in the laboratory, but seldom observed in the Earth.

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Radiation-induced chromosome aberrations in nuclear-dockyard workers

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The incidence of chromosome aberrations in peripheral blood lymphocytes of 197 dockyard workers has been followed over a 10-yr period. These workers were exposed to mixed neutron-γ radiation during the refuelling of nuclear reactors, but most exposures were below the internationally accepted maximum permissible level of 5 rem per yr. There was a significant increase in chromosome damage with increasing exposure; aberration frequency was a linear function of dose and was influenced by age and time of blood sampling after exposure.

CYTOGENETIC studies on human peripheral blood lymphocytes exposed to low doses of ionising radiation *in vitro* have shown that it is possible to detect significant levels of chromosome damage from the analysis of a few hundred cells. For example[1], the frequency of dicentric and ring aberrations in nonirradiated control blood cells was found to be 1 in 10^3 and this increased approximately 20-fold after *in vitro* exposure to 30 rad of X rays, the incidence increasing linearly over the range 0–50 rad with no evidence for a threshold dose level. At higher exposure levels, the contribution of a 'dose-squared' component becomes more significant, so that over higher ranges of exposure the yields of such aberrations may approximate to the square of dose. In contrast, the aberration frequency after *in vitro* exposure of blood cells to high linear energy transfer radiations, such as 0.7–0.9 MeV fission neutrons[2–4], increases linearly over a wide dose range and such radiations are considerably more efficient than X rays, per unit of absorbed dose, at dose levels over a range (below a few hundred rad) yielding less than an average of one dicentric aberration per cell.

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Studies on chromosomes of lymphocytes from people exposed to ionising radiation have also revealed increased aberration yields after *in vivo* exposure to relatively low doses of X rays, γ rays or fast neutrons. Moreover, because of the equivalent sensitivity of lymphocyte chromosomes to radiation damage *in vitro* and *in vivo*[5], and because of the strict relationship between aberration frequency and radiation dose and quality *in vitro*, it has become standard practice to use lymphocyte aberration frequencies as a 'biological dosimeter' in cases of accidental *in vivo* exposure to radiation[6,7]. Extrapolation from *in vitro* studies suggests that an acute X-ray exposure of 5 rad (equal to the maximum permitted annual dose for a radiation worker) would increase the spontaneous frequency of dicentric aberrations by a factor of three or four, so that the 'doubling dose' for these somatic genetic effects is quite small. However, there is little direct information on the possible effects of low dose *in vivo* exposures. A study of workers employed at a UK Atomic Energy Establishment showed that those occupationally exposed to mixed neutron-γ radiation, at levels below the maximum permissible exposure levels, had a significant excess of blood lymphocytes containing dicentric or ring aberrations relative to on-site controls[8]. However, no quantitative dose-effect relationship was evident, possibly because of inadequacy of the control data, a varying pattern of dose accumulation with time between individuals receiving similar total doses, too small a number of subjects or too few cells scored.

The population and methods of analysis

In 1968 the opening locally of an establishment for servicing and refuelling nuclear powered submarines provided an opportunity for studying a population of men subject to occupational radiation exposure within the maximum permissible limits of 5 rem per yr. As the majority of the workers at the new establishment had not received previous occupational exposure to radiation, blood samples were taken before their classification as 'radiation workers', to give background control samples, and serial blood samples were later obtained as dose was accumulated. This population has now been studied over a 10-yr period and chromosome aberrations scored in blood lymphocytes of 197 men, the majority of whom have been sampled on several occasions.

A heparinised venous blood sample of 5 ml was taken at the time of a workers 'preclassification' examination, or annual medical check up, and set up in culture later on the same day. Blood culture and chromosome preparation techniques were those that have been standard in this laboratory[9] throughout the study period, with all cultures incubated for 48 h at 37 °C. Preparations were stained with orcein and for each culture 100 cells with 45 or more centromeres were scored directly under the microscope by one observer for dicentrics, rings, acentric fragments, minutes, abnormal monocentrics, additional or absent monocentrics (aneuploidy), a medium-sized chromosome with abnormal centromere separation (almost certainly an abnormal X chromosome[10,11]) and chromatid aberrations, although not all these abnormalities would be expected to be consequences of radiation exposure (see below). All blood samples were coded and the cytological observer had no knowledge of the radiation history of any sample at the time of scoring.

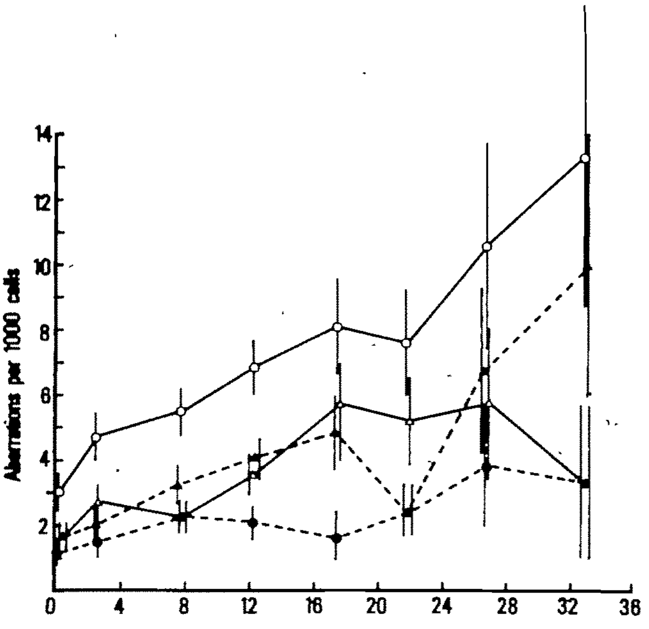

Fig. 1 Relationship between accumulated dose (rem) and mean aberration frequencies (±s.e.m.) for dicentrics (△), acentric fragments (▲), and for Cu (○) and Cs (●) cells.

Dose estimates (in rems) from film badges were provided by the Admiralty Radiation Records Centre, Institute of Naval Medicine, and were obtained independently of the receipt of blood samples and some time after completion of chromosome analyses. The radiation sources were nuclear submarine reactors undergoing refit and emitting mixed neutron-γ radiation, but the exposures were stated to involve 'almost exclusively gamma radiation'.

The data obtained over the 10-yr period were grouped by cumulative dose into 5-rem intervals and the basic data from the study are presented in Table 1 and Fig. 1. From Fig. 1 it seems that the incidence of cells containing all types of structural chromosome aberrations is positively correlated with dose. However, we have evidence from studies on a variety of populations for an increase in the spontaneous frequency of aberrations—in the absence of known radiation exposure—with increasing age (unpublished data and see also ref. 12). Also, by

Table 1 Frequencies of chromosome abnormalities in the various exposure groups

Dose range (rem)	0.0–0.9	1.0–4.9	5.0–9.9	10.0–14.9	15.0–19.9	20.0–24.9	25.0–29.9	≥30.0	Total
No. of cultures*	87	85	91	91	36	29	11	6	436
No. of individuals†	80	80	71	68	29	22	9	6	197
Total cells examined	8,700	8,500	9,200	9,075	3,700	2,900	1,040	600	43,715
Mean age (yr)	32.6	31.7	37.4	39.8	39.4	41.3	42.3	41.3	36.4
Mean dose (rem)	0.2	2.5	7.7	12.2	17.4	21.8	26.7	32.9	8.7
Mean date of culture	1971.9	1971.7	1972.1	1973.0	1973.8	1974.9	1975.0	1975.2	1972.6
Cells with additional or missing monocentrics (aneuploids)	219	247	317	305	105	98	24	13	1,328
Cells with abnormal monocentrics (Cs cells)	10	13	21	19	6	7	4	2	82
Cells with unstable aberrations (Cu cells)	26	40	50	62	30	22	11	8	249
No. of acentric elements	14	17	30	37	18	7	7	6	136
No. of dicentrics	14	23	21	32	21	15	6	2	134
No. of rings	2	6	5	4	0	3	1	0	21
No. of chromatid aberrations	156	200	199	224	73	63	30	21	966
Abnormal medium-sized (X?) chromosomes	20	7	18	29	14	8	4	2	102

* Few workers received as much as 5 rem per year so that more than one culture from an individual may occur within a given dose group

† Approximately half of the individuals had two or more cultures analysed.

Table 2 Results of multiple linear regression analysis for aberrations showing a significant dose dependence

Type of aberration	Incidence per 1,000 cells†	Increase in incidence per cell/rem $\times 10^4$ of 'total' dose‡	t	Increase in incidence per cell/rem $\times 10^4$ of 'early' dose‡	t_1	Increase in incidence per cell/rem $\times 10^4$ of 'recent' dose‡	t_2
Acentric elements	1.77(±0.42)	1.55(±0.38)	4.1***	1.20(±0.52)	2.3*	2.32(±0.91)	2.5*
Dicentrics	1.85(±0.35)	1.40(±0.38)	3.7***	1.01(±0.53)	1.9	2.32(±1.01)	2.3*
Acentric elements, dicentrics and rings combined	4.06(±0.61)	2.99(±0.56)	5.4***	2.39(±0.76)	3.1**	4.38(±1.40)	3.1**

† Baseline incidence is calculated at zero dose, mean age (36.4 yr) and mean date of culture (1972.6).
‡ These are equivalent to the coefficients b, b_1 and b_2 of the model described in the text.
*, **, ***, Significance levels at 0.05, 0.01 and 0.001 respectively.

the very nature of the dose accumulation process, it is to be expected that older workers and samples from later years would contribute disproportionately to the higher dose categories. The positive correlation between dose and age, and dose and year of culture, and aberration frequency is shown in Fig. 2 and it is clearly necessary to take account of the influence of these two factors in examining for correlations between level of exposure and aberration yield. For this reason the data were initially analysed by multiple linear regression under the assumption that n_i, the number of aberrations in the i-th culture, is Poisson-distributed with mean μ_i given by

$$\mu_i = N_i(a + bx_i + cy_i + dz_i)$$

where N_i = number of cells examined in i-th culture; x_i = cumulative dose received by the blood donor at the time of the i-th culture; y_i = age of donor at the time of culture; and z_i = year in which sample for the i-th culture was taken. We accept the conventional assumption of a Poisson distribution, but note that the small numbers of aberrations give insufficient data for a rigorous test of this premise. The parameters were estimated by maximising the likelihood, the significance of an effect being judged by standard likelihood-ratio tests. In this way a 'best linear model' containing only the significant ($P \leqslant 0.05$) effects was found for each type of aberration. Those aberrations whose 'best linear model' included a dose effect were then further analysed in terms of: (1) a model with a term x_i^2 to see whether significant second-order effects of dose were present; (2) a model with a term x_iy_i added to see whether a significant age–dose interaction was present.

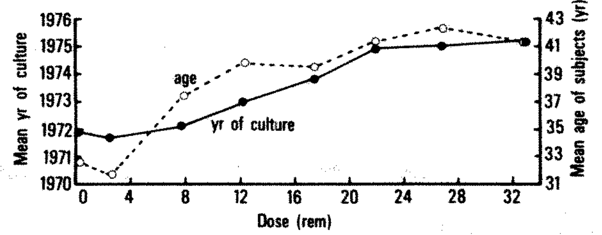

Fig. 2 The relationships between accumulated dose (rem) and year of culture (●), and dose and age of blood donor (○).

Studies on radiotherapy patients and on individuals accidentally exposed to high doses of radiation have shown that the frequency of unstable aberrations in peripheral blood lymphocytes, such as dicentrics, rings and fragments, decreases in the months and years following exposure[13]. Thus, for a given time of blood sampling, an exposure a few days or weeks before sampling results in a higher detected incidence of induced unstable aberrations than an equivalent exposure received a year or more before sampling. For this reason a third model with terms $b_1x_{1i} + b_2x_{2i}$ replacing bx_i in the best linear model was also considered, where x_{1i} = cumulative dose received before the beginning of the second full calendar year preceding the date of culture, and $x_{2i} = x_i - x_{1i}$ = the dose received since that time. The aim of this third approach was therefore to establish whether the frequency of a given aberration type showed a stronger dependence on the dose received recently (x_{2i}) than on that received earlier (x_{1i}), as would be expected if there were a genuine dose–effect causal relationship.

Dependence of aberration incidence on dose, age and time of exposure

These analyses to take into account the contribution of age and date of culture revealed significant effects of dose for the incidence of dicentric aberrations, acentric fragments and cells with such unstable aberrations (Cu cells), but not for cells with symmetrical rearrangements (Cs cells); these data are summarised in Table 2. *In vitro* radiation 'model' experiments and 'high dose' *in vivo* studies have clearly shown that the incidence of dicentric (±ring) aberrations, acentric fragments and total cells with such unstable aberrations (Cu cells) is dose dependent and that these aberrations are typical radiation-induced changes: the difficulty of detecting symmetrical rearrangements contributes to the much lower incidence of Cs relative to Cu cells and to the diminished dose dependence of Cs cell types. In contrast, as most peripheral blood lymphocytes are in a G1 phase when exposed to radiation, the majority of chromatid-type aberrations observed in such cells are not radiation-induced and these aberrations, and the incidence of aneuploidy in first post-irradiation mitoses, show little dependence on dose. Similarly, the incidence of a medium-sized chromosome with abnormally separated chromatids is independent of radiation exposure, but is heavily dependent on age and sex, being prevalent in ageing females and identified as an X chromosome[10,11]. In the present data a significant age–effect relationship, but no dose–effect relationship, was found for aneuploid cells, for chromatid aberrations and for 'X chromosomes' with abnormal centromere separation, so that these results are as expected.

The above analyses suggest that the asymmetrical, or unstable, aberrations observed in the blood cells of these dockyard workers are a consequence of their exposure to radiation. Detailed analysis of these aberrations further reveals that: (1) there are no detectable second-order effects of dose—all data are consistent with a linear dose response; (2) in all categories there is a positive age–dose interaction (Table 3), indicating a greater susceptibility to radiation-induced chromosome damage with increasing age—as suggested by earlier *in vitro* studies[14,15]; this was statistically significant ($P < 0.05$) for dicentric aberrations; and (3) for all categories the dependence on 'recent' dose is greater than on 'early' dose, although in no case do the coefficients b_1 and b_2 differ significantly (Table 2).

Implications of the findings

The demonstration of a significant dose-dependent increase in chromosome aberrations in peripheral blood leukocyte chromosomes in a population of closely monitored nuclear-dockyard workers, subject to occupational radiation exposure

Table 3 Age–dose interactions for aberrations with a significant dose-dependence

	Significance level of interaction effect	Estimated increase in incidence per rem per cell ($\times 10^4$)		
		Age 25 yr	Age 40 yr	Age 55 yr
Acentric elelements	$P = 0.24$	1.04(±0.59)	1.64(±0.40)	2.25(±0.71)
Dicentrics	$P = 0.030$	0.53(±0.59)	1.57(±0.38)	2.60(±0.68)
Acentric elements, dicentrics and rings combined	$P = 0.021$	1.57(±0.85)	3.27(±0.57)	4.97(±0.99)

within the maximum permissible level of 5 rem per yr, raises a number of questions. We should perhaps first note that the estimated rate of increase of dicentric aberrations with dose (1.4×10^{-4} dicentrics per cell per rem) is of a similar order to that obtained from our earlier data[8] on a population of occupationally exposed personnel at UKAEA Windscale, although the number of cells examined in the Windscale study was insufficient to establish significance. In this context it should be noted that the effect of increasing dose is not seen in successive cultures from the same individual, at the exposure levels encountered by our population, because the number of cells examined per individual is insufficient. For example, in these conditions of exposure, the detection of the estimated effect of 10 rem on the incidence of dicentrics in a single individual would necessitate examining of the order of 10,000 cells—a formidable task.

The yield of dicentric aberrations, or of acentric fragments, in cells from individuals obtained before occupational exposure, or after exposure to <1 rem, was ~ 1 in 700 cells, rising approximately fourfold after accumulation of doses of 20 to 30 rem. The observed increase is therefore not large, but it is a direct expression of increased genetic damage caused by radiation exposure. The dicentric aberrations observed are frequently cell lethal, as also are a proportion of the free fragments, but some of the cells containing acentric fragments, and those with symmetrical rearrangements such as translocations, are viable and a proportion of these are detected as Cs cells. Cs cells are not easily distinguished, represent ~ 1 in 900 cells studied in individuals receiving <1 rem, and are approximately three times as frequent in individuals who accumulated more than 25 rem. It is not clear whether there is a somatic effect on the individual as a result of the presence of Cs cells, although each such cell presumably contains some kind of mutational change and there are many thousands of such cells present in blood lymphocytes and bone-marrow cells of individuals not exposed to radiation. Our data therefore tell us nothing about possible biological consequences, but merely demonstrate that it is possible to detect a biological effect at the chromosome level of occupational exposure to ionising radiation below the internationally agreed maximum permissible levels.

There has been much recent publicity concerning possible increased cancer incidence—particularly leukaemias and lymphomas—in occupationally exposed personnel in the USA in a Naval dockyard[16,17] and in the atomic energy plant at Hanford[18,19]. However, the data on which some of these claims have been based, and the analyses involved, have been very heavily criticised[20–23]. It is therefore pertinent for us to point out that the exposed population that we have studied is small, subject to very low levels of radiation exposure for periods up to only 10 yr—and is therefore unlikely to provide useful data on the incidence of malignant disease. During the 10-yr study there were 2 deaths amongst the 197 men studied, both due to myocardial infarction: there was no record of deaths due to leukaemia or other malignant disease. Examination of the Registrar General's returns for deaths attributed to, or associated with, leukaemias for the area which includes our naval dockyard, has revealed no increase in the incidence of leukaemia relative to other populations over the years 1967–77, and no disproportionate number of dockyard workers suffering such deaths. We emphasise again, however, that the population size is small and it would seem sensible to monitor causes of death in a larger population of workers exposed to radiations within the occupational limits—a project that has already been initiated by the National Radiological Protection Board[24].

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Selective activation of human β- but not γ-globin gene in human fibroblast × mouse erythroleukaemia cell hybrids

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The human α- and β-globin genes have been activated in MEL × human fibroblast cell hybrids. However, even though the human γ- and β-globin genes are closely linked and were shown in these hybrid clones to be present in approximately equal numbers, no human γ-globin mRNA was produced. Thus, the human β- and γ-globin genes in these cells are differentially regulated apparently by a positive regulatory factor(s) specific for individual globin genes.

IN order to define the mechanism of globin gene regulation in mammalian cells, we have examined the expression of the mouse and human haemoglobin genes in somatic cell hybrids between mouse erythroleukaemia (MEL) cells and various human cell lines[1–6]. Previous studies have shown that fusions between MEL cells and human (or Chinese hamster) erythroid cells resulted in co-expression of mouse and human (or Chinese hamster) haemoglobin[1,2]; fusions of MEL cells with non-erythroid cells resulted in hybrids which could not be induced to make haemoglobin[3,4,7–10], even though the globin genes were present in the hybrid cells[3]. Gopalakrishnan *et al.*[4] obtained evidence for the existence, in the cytoplasm of non-erythroid cells, of a

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Table 1 Phenotype of 2S MEL × HLN hybrids and parents

Cell line	Chromosomes			Isozymes				
			%	LDH			APRT	
Parents	Mean	Range	Meta-centrics	M	H-A	H-B	M	H
2S MEL	57.3	44–75	10.3	+++	0	0	+++	0
HLN	46.1	44–48	88.6	0	+++	+++	0	+++
Hybrids								
XVIII-5	68.0	51–86	17.1	+++	++	+++	+++	0
XVIII-15	55.9	42–74	11.5	+++	+++	0	+++	0
XIX-2	122.3	73–159	22.2	+++	+	+++	+++	±
XIX-7	72.1	60–89	20.3	+++	++	0	+++	0
XIX-10	74.4	59–90	24.4	+++	+	0	+++	±
XX-8	66.4	46–84	13.7	+++	++	++	+++	0

2S MEL is an MEL cell line containing a sub-tetraploid complement of chromosomes[5]; it is TK$^-$. HLN stands for a human Lesch-Nyhan skin fibroblast primary cell line (CRL 1112, ATCC), and is HGPRT$^-$. XVIII-5 is the 5th clone of the 18th fusion of 2S MEL cells to human non-erythroid cells. For the chromosome analysis: approximately 10^7 cells were washed in Dulbecco's phosphate-buffered saline, swollen in 0.075 M KCl, fixed in 3 : 1 methanol–acetic acid and dropped onto glass slides. Photographs of at least 15 metaphase spreads per clone were counted to estimate the total number of chromosomes and per cent of metacentric chromosomes. The spreads were made from an aliquot of the same expanded and induced cell population from which the isozyme analyses were done and from which the RNA and DNA were isolated for the studies in Table 2 and Figures 2–4. For the isozyme analysis: human chromosome 11 is known to have LDH-A and the γ-δ-β globin gene complex[20]; human chromosome 16 is known to have APRT and human α-globin[19]; LDH-B is on human chromosome 12. APRT analyses were by the method of Tischfield *et al.*[30]. Only hybrids positive for human LDH-A (chromosome 11) are shown; approximately 25% of the successful fusions between 2S MEL and HLN cells resulted in retention of chromosome 11 after expansion. M, mouse; H-A, human LDH-A; H-B, human LDH-B; H, human. The scale is: 0, None detectable; ± → +++ = increasingly positive.

negative regulatory factor which prevented the subsequent induction of haemoglobin in MEL 'cybrids' obtained by fusion of intact MEL cells with enucleated non-erythroid cells. Recently, Deisseroth and Hendrick[11] showed that human α-globin mRNA as well as mouse globin mRNA was expressed in 1S MEL × human erythroid cell hybrids when human chromosome 16 had been selectively retained. It is not known if a fibroblast α-globin gene would have been activated in these 1S MEL hybrids.

We wish to learn if erythroid cells (such as MEL cells) might contain one or more positive regulatory factors. Evidence for positive regulation would exist if conditions could be found that result in activation of fibroblast globin genes (as well as MEL globin genes) in MEL × fibroblast hybrids. A gene dosage effect has been used by others to obtain phenotypic expression of differentiated functions in somatic cell hybrids[12–17]; an increased input of chromosomes from one parental cell can yield hybrid cells in which a previously unexpressed function might be activated[17,18]. Weiss and coworkers[17] have extensively studied the activation of albumin synthesis and other liver functions in rat hepatoma × mouse lymphoid cell hybrids. The expression of rat hepatic functions, and the activation of such functions from the mouse lymphoid chromosomes, was greatly increased by the use of a 2S (tetraploid) hepatoma parental cell[17]. Our laboratory developed a stable 2S (tetraploid) MEL cell line[5] and demonstrated that 2S MEL × fibroblast hybrids were inducible for haemoglobin as shown by positive benzidine staining of hybrid cell clones. It was also demonstrated that 1S MEL × fibroblast fusions can result in haemoglobin inducible hybrids although the efficiency is greatly increased when 2S MEL cells are used.

We have now tested for the expression of mouse and human globin genes by measuring the amounts of mouse and human globin mRNA in somatic cell hybrids derived from fusions of 2S MEL cells with human fibroblasts. The human α-globin gene is on chromosome 16 (ref. 19) and the human γ-δ-β globin gene locus is on chromosome 11 (ref. 20). Adults synthesise primarily haemoglobin A ($\alpha_2\beta_2$) while the human fetus synthesises primarily haemoglobin F ($\alpha_2\gamma_2$). Soon after birth, haemoglobin F is replaced by haemoglobin A. Since the γ- and β-globin genes are closely linked, our experimental model is unique in that it provides the opportunity to examine the expression of two closely linked genes at the molecular (mRNA) level. Our results indicate that, when human chromosome 16 is present in the hybrid clone, human α-globin mRNA is produced (in addition to mouse globin mRNA); when chromosome 11 is present, human β-globin mRNA is found. Surprisingly, however, even when human β-globin mRNA is present in large amounts, no human γ-globin mRNA is detectable. Thus, even though the β- and γ-globin genes are closely linked and were shown to be present in approximately equal numbers in these hybrid cells, only the β-globin gene is expressed. Thus, the mechanism of differential regulation of human β- and γ-globin genes seems to involve, at least in these cells, a globin gene specific regulatory factor(s).

Hybrids between MEL cells and human fibroblasts

Fusions were performed between thymidine kinase deficient (TK$^-$) 2S MEL cells[5] and various hypoxanthine-guanine-phosphoribosyl-transferase deficient (HGPRT$^-$) human non-erythroid cell lines. In each fusion, several individual clones were grown to 10^6 cells and analysed for human lactate dehydrogenase-A (LDH-A); human chromosome 11 contains LDH-A and the γ-δ-β globin gene complex. Positive clones were expanded to 5×10^8 cells and a repeat isozyme analysis carried out. If the clone was still positive for LDH-A, a portion of the

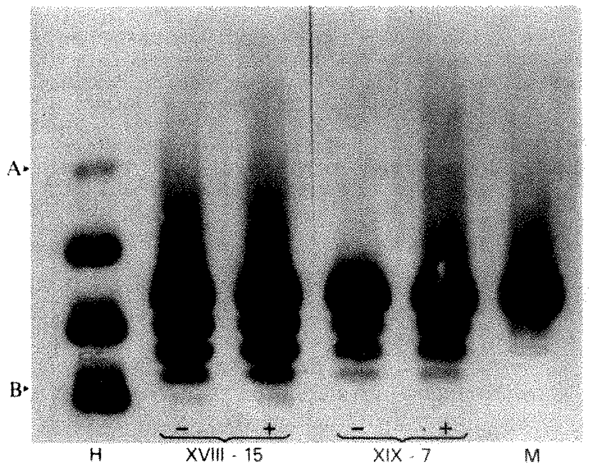

Fig. 1 Lactate dehydrogenase (LDH) isozyme pattern of the parental 2S MEL cells, human Lesch-Nyhan (HLN) skin fibroblast primary cells, and two of the hybrids between them. The parental lines, 2S MEL and human Lesch-Nyhan fibroblasts (see Table 1), were grown in F14 medium (available without $NaHCO_3$ from Gibco as F12-modified medium 72004) supplemented with 3.7 gl^{-1} $NaHCO_3$ and with 10% fetal calf serum[31]. 2S MEL cells (1×10^7) were mixed with 1×10^6 trypsinised HLN fibroblasts, washed twice in serum-free medium, resuspended in 1 ml of serum-free medium containing 250 HAU of inactivated Sendai virus, incubated at 0 °C for 15 min, then at 37 °C for 1 h, resuspended at 5×10^4 cells ml^{-1} in medium with serum, and incubated at 37 °C in 10% CO_2 at 100% humidity[5]. The next day cells were plated in 0.1 ml in microtiter wells (Falcon) at different dilutions. After 24 h, 0.1 ml of medium containing 2 × HAT was added to each well. After 2–3 weeks, wells containing visible colonies (all floating cells) were transferred to 60-mm dishes in medium containing 10^{-4} M hypoxanthine and 1.6×10^{-5} M thymidine. After 1 week in this medium, the cells were transferred to regular growth medium (F14 plus 10% fetal calf serum) and expanded to 1×10^6 cells. Each clone was then analysed for human LDH-A. Electrophoresis was carried out using the Gelman LDH isozyme electrophoresis system (according to Technical Bulletin 23) with Corning ACI agarose electrophoresis film. Positive clones were grown to $3\text{–}5 \times 10^8$ cells and re-checked for LDH-A. One-half the culture was transferred to medium containing 2.0% DMSO and incubated for 5–6 days (reaching approximately 10^9 cells); the remaining half was continued in regular growth medium for 2–4 days to reach 10^9 cells. Aliquots from each incubation were taken for LDH and APRT isozyme electrophoresis, chromosome analysis, benzidine reactivity and ^{3}H-leucine incorporation into globin chains. All remaining cells were used for RNA and DNA extraction as described in the legend to Fig. 2. H, human Lesch-Nyhan fibroblasts; M, 2S MEL cells; XVIII-15 and XIX-7 are hybrids; –, means after expansion to 10^9 cells but without induction; +, means after expansion and induction of haemoglobin by 5–6 days growth in 2% dimethylsulfoxide. A, human LDH-A tetramer; B, human LDH-B tetramer.

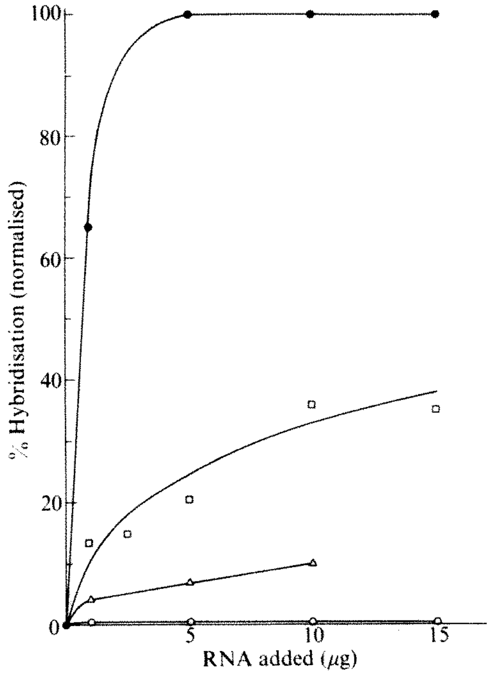

Fig. 2 Hybridisation of hybrid cell RNA to ^{3}H-mouse globin cDNA and ^{32}P-human α-globin cDNA. Hybrid cells (see Fig. 1 legend) were pelleted, washed once in minimal essential medium, repelleted, and then suspended in a solution of 10 mM Tris-HCl, *p*H 8, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.1% Triton X-100, 0.5 mM dithiothreitol, and 0.1 M sucrose. Nuclei were obtained by homogenisation with a motor driven Teflon-glass Dounce homogeniser (4–6 strokes at full speed); the nuclear suspension was diluted 1 : 1 in an identical buffer but with a sucrose concentration of 0.25 M. 20 ml of the nuclei suspension was then layered onto 10 ml of a 0.33 M sucrose cushion containing 10 mM Tris-HCl, *p*H 8, 5 mM $MgCl_2$, and 0.5 mM dithiothreitol. The nuclei were pelleted by centrifugation at 800 *g* (2,200 r.p.m. in a GLC tabletop centrifuge) for 5–10 min. The supernatant fraction was removed for RNA extraction; DNA was extracted from the nuclear pellet as previously described[26]. For RNA extraction, the supernatant from the preparation of nuclei was made 0.1 M in NaOAc (*p*H 5) and 10 mM EDTA. Subsequent phenol extraction at 55 °C, ethanol precipitation, DNase treatment, re-extraction with phenol, and passage over a G-50 Sephadex column was as described previously[32,33]. Mouse globin cDNA was prepared as previously described[34] using mouse reticulocyte mRNA prepared from polysomes[32] as a template for RNA-directed DNA polymerase. ^{3}H-CTP was used as the label yielding a probe with a specific activity of 10,000–12,000 c.p.m. μg^{-1}. A size fraction 500–600 nucleotides in length was selected by alkaline sucrose gradient centrifugation[34]. Plasmid DNA (100–150 μg) from JW 101 (human α), JW 102 (human β), or JW 151 (human γ)[21] was digested with *Mbo*II (JW 101) or *Hha*I (JW 102, JW 151) and the resulting DNA fragments were fractionated on a sucrose gradient (5–20%) in 10 mM Tris-HCl, *p*H 7.5, 100 mM NaCl, and 1 mM EDTA[35]. The largest fragment, which contains the globin gene insert, was recovered by ethanol precipitation. Nick-translation[36,37] in the presence of ^{32}P-dCTP resulted in a specific activity of approximately 10^8 c.p.m. μg^{-1}. Each plasmid fragment was annealed in 50% formamide, 0.5 M NaCl to homologous mRNA (that is, the fragment containing either the α- or β-globin gene was annealed to human adult globin mRNA (α and β) while the *Hha*I fragment containing the γ-globin gene was annealed to fetal reticulocyte mRNA (α, β, and γ)) at 66.5 °C. At this temperature DNA will not reanneal, but a duplex between mRNA and the cDNA strand of the insert is formed. Digestion with S_1 nuclease resulted in destruction of all DNA except that in a duplex. The single-stranded cDNA fragment was then recovered by alkaline sucrose gradient centrifugation[35] and used for mRNA · cDNA (and DNA · cDNA) hybridisations. mRNA · cDNA hybridisation was carried out as previously described[34] except that both ^{3}H-mouse globin cDNA and ^{32}P-human α-globin cDNA were used in each double labelled reaction. Titrations were performed in 50% formamide, 0.5 M NaCl at 66.5 °C. Incubation to achieve equilibrium was for 48 h after which duplex formation was analysed by S_1 nuclease resistance[33]. Each 10 μl reaction mixture contained 2,000 c.p.m. of ^{3}H-mouse globin cDNA and 2,000 c.p.m. of ^{32}P-human α-globin probe. Control incubations showed: (1) duplex formation resulting from titration with mouse reticulocyte mRNA reached 82% for the ^{3}H-mouse cDNA, but less than 1% for the ^{32}P-human α cDNA; and (2), duplex formation resulting from titration with human reticulocyte mRNA reached 71% for the ^{32}P-human α cDNA but less than 1% for the ^{3}H-mouse cDNA. Values were normalised by dividing each ^{3}H value by 0.82 and each ^{32}P value by 0.71. The ^{3}H values for all six hybrids were similar and were, therefore, averaged. Similar computations were performed for the data in Figs 3 and 4. ●, Hybridisation with ^{3}H-mouse globin cDNA (the values for all six hybrid cell RNAs have been averaged and normalized; see above); □, hybridisation of the RNA from hybrid XIX-2 with ^{32}P-human α-globin cDNA; △, RNA from XIX-10 with ^{32}P-human α-globin cDNA; ○, RNA from XVIII-5, XVIII-15, XIX-7, and XX-8 with ^{32}P-human α-globin cDNA.

cells were grown in 2% dimethylsulphoxide (DMSO) for 5–6 days and the remainder for a shorter period without inducer (see Fig. 1 legend). Aliquots from each incubation were taken for isozyme analysis, karyology, haemoglobin inducibility and ^{3}H-leucine incorporation into globin chains. RNA and DNA were then extracted from the remaining cells as described in Fig. 2 legend.

The phenotypic characteristics of the 2S MEL and the human Lesch-Nyhan (HLN) skin fibroblast primary parents, as well as of six hybrids between them, are shown in Table 1. Only the six hybrids which are positive for LDH-A (Fig. 1) are shown, since β-globin mRNA was not found in the hybrid clones lacking LDH-A (data not shown). APRT was examined as it is a marker for chromosome 16 which carries the human α-globin gene. Chromosome analysis shows that the hybrids possess an increased number of chromosomes and a higher percentage of metacentrics than the 2S MEL parent. All six were positive for mouse LDH, mouse APRT, and human LDH-A; three were positive for LDH-B (carried on chromosome 12); two for human APRT. XIX-2, the hybrid with the highest number of chromosomes (122 with 22% metacentrics), was the only clone positive for all three human enzymes: LDH-A, LDH-B, and APRT. As shown in Table 2, all six hybrids could be induced to make haemoglobin as determined by benzidine staining.

Detection of human globin mRNA in cell hybrids

Pure single stranded probes complementary to human α-, β-, or γ-globin mRNA were prepared from recombinant cDNA plasmid clones JW 101, 102, or 151 (ref. 21), respectively, as described in Fig. 2 legend. The cytoplasmic RNA from each hybrid line was separately hybridised to each ^{32}P-labelled human probe in the presence of ^{3}H-labelled mouse globin cDNA. In the data in Figs 2–4, the ^{3}H-mouse globin cDNA hybridisation values for all six hybrid cell RNAs were averaged (solid circles), and all points were normalised, as described in Fig. 2 legend. Thus, the plots of α, β, and γ human globin mRNA for each of the six hybrids can be compared to each other.

As shown in Fig. 2, two hybrids contain small amounts of human α-globin mRNA: XIX-2 and XIX-10. Both these hybrids were positive for human APRT on enzyme analysis (Table 1). Five of the six hybrids positive for human LDH-A had clearly detectable human β-globin mRNA (Fig. 3). XX-8, the hybrid with the highest concentration of human β-globin mRNA and a strong LDH-A band, had 35% as many human β-globin mRNA sequences as mouse globin mRNA sequences. There is a fairly close correlation between the amount of LDH-A in a given hybrid cell line (Table 1) and the amount of human β-globin mRNA present (Fig. 3, Table 2).

None of the hybrids had detectable human γ-globin mRNA sequences, even at 60 μg RNA input (Fig. 4). When the human γ-globin cDNA probe was annealed to mRNA isolated from fetal cord blood in a simultaneous experiment, 72% of the γ probe was incorporated into duplexes; a saturation plateau was achieved above an input of 5 ng of partially purified mRNA (data not shown). As expected, since the γ- and β-globin genes are closely linked, DNA·cDNA hybridisation demonstrated approximately equal concentrations of γ- and β-globin gene sequences in the hybrid cell DNA (data not shown). Analysis of the RNA from the expanded but uninduced hybrid cell showed very low levels of mouse globin mRNA, no (or barely detectable) human β-globin mRNA, and no detectable human γ-globin mRNA.

Carboxymethylcellulose chromatography of the ^{3}H-leucine globin chains synthesised by the hybrids positive for human β-globin mRNA revealed a peak in the region of human β-globin. However, the calculated number of globin chains in the

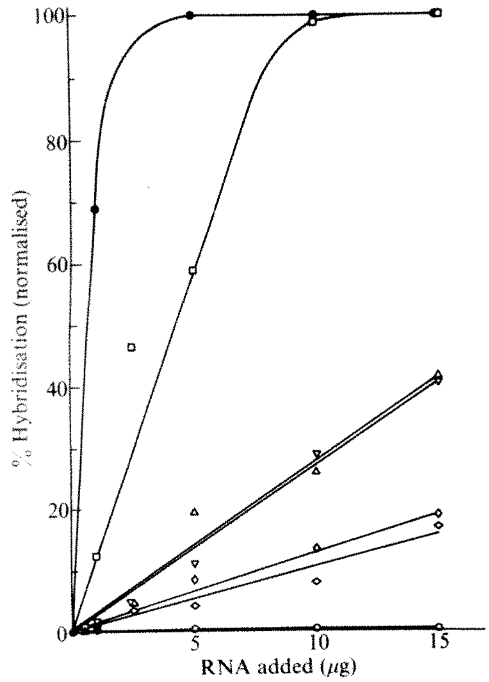

Fig. 3 Hybridisation of hybrid cell RNA to ^{3}H-mouse globin cDNA and ^{32}P-human β-globin cDNA. Experimental conditions were as for Fig. 2 except that human β-globin cDNA from the cDNA plasmid JW 102[21] was used. Hybridisation of the β-globin probe to globin mRNA obtained from adult human reticulocytes reached a plateau of 78.5%. All hybrid RNA points with ^{32}P-human β cDNA were divided by 0.785 to normalise them to a maximum of 100%. ●, Average value for hybrid cell RNA with ^{3}H-mouse globin cDNA; open symbols are for hybrid cell RNA with ^{32}P-human β-globin cDNA; □, XX-8; △, XIX-7, ▽, XVIII-15; ◇, XIX-2; ◇, XVIII-5; ○, XIX-10.

human β-globin region did not correlate with the concentration of human β-globin mRNA molecules known to be present. Further analysis of the putative human β-globin is underway.

Discussion

In this study, we have shown that the globin genes of human fibroblasts can be activated in fibroblast × 2S MEL cell hybrids. The expression of fibroblast globin genes implies the presence in the induced 2S MEL hybrid cells of a positive regulatory factor of some type. While the adult human globin genes, α and β, were consistently activated when the human chromosomes bearing these genes were present in the hybrid clones (chromosome 16 for α, chromosome 11 for β), the human γ-globin gene, which is closely linked with β-globin, was not expressed at all. Thus, in these cells, there is a differential regulation within the γ-β globin gene locus.

Several mechanisms might account for such differential gene regulation. First, a diffusable regulatory factor could act as a positive activator of the human β- but not γ-globin gene by binding to a controlling region adjacent to the globin gene structural sequences. Many examples of such regulatory factors in prokaryotes have been described[22]. A single factor might be responsible for activating the human α, human β, and the mouse globin genes, or several different globin gene specific factors could be involved.

A second possible mechanism which could account for differential globin gene activation in these hybrid cells would be the state of the parental cell's chromatin before and after fusion. Expression of a gene requires that its sequences be in an active conformation in nuclear chromatin. Exposure of nuclei to pancreatic DNase I results in selective digestion of active genes[23–26]. Thus, in erythroid cells, the globin genes are DNase I sensitive while in fibroblasts the globin genes are resistant. We assume that the human β- and α-globin genes were resistant in the parental fibroblasts but were converted to the active conformation in the hybrid clones. It is thought that non-histone proteins may be operative in initiating and propagating the active conformation of nucleosomes associated with expressed genes. If so, these 'regulatory factors' for mouse MEL cells would act on both mouse and human α- and β-globin genes, but possibly not on the human γ-globin gene. Since adult human fibroblasts were used in this study, it is possible that, if chromatin structure of the parental cells is important, a fusion of 2S MEL cells with fetal fibroblasts would result in expression of human γ-globin mRNA. To test this hypothesis, hybrids were formed between 2S MEL and fetal amniotic cells and are now being analysed for β- and γ-globin mRNA synthesis.

Table 2 Haemoglobin inducibility of 2S MEL × HLN hybrids and parents

Cell line	Haemoglobin inducible (%)	Mouse globin mRNA	Human globin mRNA α	β	γ
Parents					
2S MEL	60	+++	0	0	0
HLN	0	0	0	0	0
Hybrids					
XVIII-5	85	+++	0	+	0
XVIII-15	40	+++	0	++	0
XIX-2	55	+++	++	+	0
XIX-7	85	+++	0	++	0
XIX-10	70	+++	+	0	0
XX-8	95	+++	0	+++	0

Haemoglobin inducibility is the per cent of cells in the population which were benzidine positive after incubation for 5 days in 2% dimethylsulphoxide (DMSO). Actively growing floating cells were resuspended at a concentration of 0.5–1 × 10^5 cells/ml in F14 medium containing 10% fetal calf serum and 2% DMSO. After 5 days the cells were stained by the addition of one-tenth the volume of a fresh solution containing 0.2% (w/v) of benzidine dihydrochloride and 0.4% (v/v) H_2O_2 in 0.5 M acetic acid[1]. Mouse globin mRNA was measured by hybridisation with ^{3}H-cDNA made from unfractionated mouse reticulocyte mRNA; human α-, β- and γ-globin mRNA were measured with pure probes obtained from recombinant cDNA plasmids (see Fig. 2 legend). The scale is: 0 = none detectable; + → +++ = increasingly positive.

Another possible mechanism of differential gene expression is selective processing of globin gene transcripts. In our present

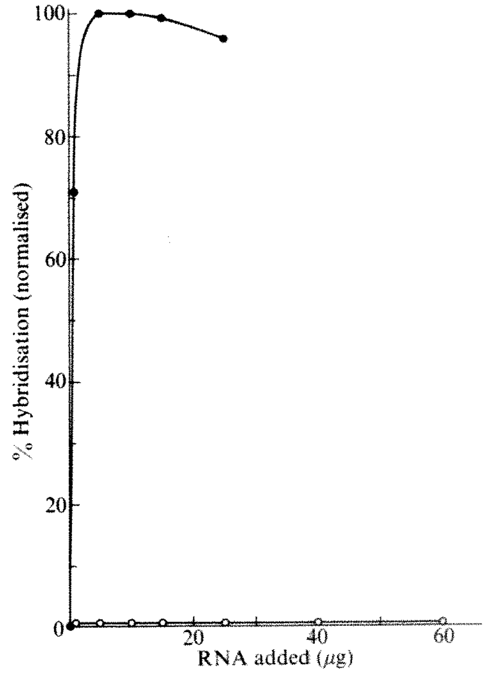

Fig. 4 Hybridisation of hybrid cell RNA to ^{3}H-mouse globin cDNA and ^{32}P-human γ-globin cDNA. Experimental conditions were as for Fig. 2, except that human γ-globin cDNA from the cDNA plasmid JW 151[21] was used. Hybridisation of the γ-globin probe to globin mRNA obtained from cord blood red cells reached a plateau of 72%. ●, Average value for hybrid cell RNA with ^{3}H-mouse globin cDNA; ○, hybrid cell RNA with ^{32}P-human γ-globin cDNA (all six were negative).

study, the hybrid cell nuclei were used for DNA extraction, and the RNA was isolated from the cytoplasm only. It is possible that human γ-globin gene sequences were present in the nuclei, but were selectively degraded or were not processed in such a way that they could reach the cytoplasm. Thus, specific and selective MEL processing enzymes could have been involved. Extraction and analysis of nuclear RNA should detect all but rapidly degraded γ-globin gene sequences.

Whether the factor(s) that controls globin gene expression acts by binding directly to a controlling region of a globin gene 'operon', at the level of chromatin structure, by influencing processing of the primary globin gene transcript, or by some other mechanism, there is no factor in the induced MEL hybrid cell that results in expression of the human γ-globin gene.

If a diffusable positive regulatory factor(s) does exist in induced MEL cells, it should be possible to isolate and characterise it. The recent availability of cloned globin genes from genomic DNA obtained by recombinant DNA technology[27,28] should allow cell-free systems to be developed to test the mechanism of action of these factors. As a preliminary to cell-free studies, we are attempting to isolate such putative factors by fractionating the induced 2S MEL cells and microinjecting the cell fractions into uninduced MEL cells to look for induction[6,29]. Preliminary results indicate that a 2S MEL cell can be induced by microinjecting 10^{-11} ml of 100 mM hexamethylene bisactamide (W. F. A. and E. Diacumakos, unpublished results). Microinjection of MEL cell fractions is now underway.

The existence of a factor specific for human β-globin implies that a similar factor might exist to control the human γ-globin gene. Such a factor might have clinical importance since the ability to express the γ-globin in β-haemoglobinopathies or in β-thalassemia could be highly beneficial. To this end, hybrids are also being made using fibroblasts from patients (and at-risk fetuses) with sickle cell anaemia and β-thalassemia to study the globin expression from fibroblasts of patients with beta chain abnormalities.

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Escherichia coli mutants accumulating the precursor of a secreted protein in the cytoplasm

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The maltose binding protein of Escherichia coli *is secreted into the external periplasmic compartment of the cell. This article describes a selection procedure for the isolation of mutants which fail to export this protein. These mutants probably result from alterations in the amino terminal 'signal sequence', causing the maltose binding protein produced to accumulate in the cytoplasm in its precursor form.*

SECRETED proteins in both bacterial and eukaryotic cells are initially synthesised as longer precursors with so-called 'signal sequences' at their amino termini, which are subsequently excised[1–6]. This signal sequence is thought to be responsible for initiating passage of the polypeptide through the membrane while it is still a nascent chain attached to the ribosome. The study of protein secretion in bacteria provides the obvious advantages of an easily-manipulated genetic system. We describe here the isolation of mutants of *Escherichia coli* in which the maltose binding protein (MBP), which is normally secreted across the cytoplasmic membrane into the periplasmic space, accumulates in the cytoplasm in its precursor form. This is most probably a result of alterations in the signal sequence. The MBP is a key component of the maltose transport system of this organism. It is a 38,000 molecular weight protein[7] which is synthesised on ribosomes attached to the cytoplasmic side of the cytoplasmic membrane[8]. As predicted by the signal hypothesis, MBP is initially synthesised with an amino-terminal extension some 20 to 30 amino acids long[9].

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Fusions of *malE* and *lacZ* genes

To isolate mutants in the MBP (*malE* gene), we used the gene fusion technique of Casadaban[10,11] to isolate strains in which the *lacZ* gene encoding the normally cytoplasmic enzyme β-galactosidase is fused to the *malE* gene. The product of such a fusion is often a hybrid protein in which the amino terminus of β-galactosidase has been replaced with an amino-terminal MBP sequence but which retains β-galactosidase enzyme activity. The signal hypothesis predicts that such a hybrid protein would be exported to the periplasm if it includes an intact MBP signal sequence. As described below, the properties of such fusion strains permit a direct selection for signal sequence mutants.

Strain PB72-47 is a *malE–lacZ* fusion strain which synthesises a very large hybrid protein (molecular weight 131,000) including a major amino-terminal portion of the MBP (Fig. 1). This strain does not, however, export the hybrid protein to the periplasm. Instead, it is localised to the cytoplasmic membrane (Table 1), suggesting that the export process may at least have been initiated. The strain also exhibits a rather unusual phenotype. When it is induced for the synthesis of the hybrid protein by the addition of maltose to the growth medium, cell division is inhibited concomitantly with the appearance of β-galactosidase activity. The cells at this point elongate to several times their normal length. Eventually, 3 to 4 h after induction, the cells begin to lyse. This 'maltose-sensitive' phenotype is a direct consequence of the synthesis of the hybrid protein. Mutations which eliminate the synthesis of hybrid protein (such as chain-terminating nonsense mutations in the hybrid gene) restore normal growth. The maltose-sensitive phenotype of strain PB72-47 apparently results from the cell's abortive attempt to export the hybrid proteins. For some reason, the cell's export apparatus cannot accommodate these unusually large polypeptides, and they remain literally 'stuck' in the cytoplasmic membrane. This has adverse consequences for the cell, since the failure to export these hybrid proteins also interferes with the transport of other exported proteins, which accumulate within the cell in their precursor forms[12].

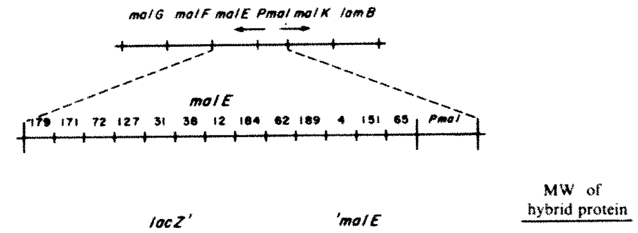

Fig. 1 The *malE* gene is one of five genes whose products are components of the maltose transport system. These are organised into two operons orientated in opposite directions and which diverge from the promotor region designated here as 'Pmal'[16]. Maltose is the inducer for both operons. A deletion map of the *malEFG* operon has been constructed. The *malE* gene has been divided into 13 segments, so identified on the basis of recombination of deletions with insertions of phage Mu DNA at different points along its length (indicated here by numbers). Using these same Mu insertions, the approximate portion of the *malE* gene included in the hybrid gene in the *malE–lacZ* fusion strains PB63–37 and PB72–47 has been established. The end point within the *lacZ* gene for both fusions is genetically identical. The genetic data agrees with the molecular weights determined for the hybrid proteins encoded by these two fusion strains. Thus, the molecular weight differences discerned between different *malE–lacZ* hybrid proteins reflect differences in the contribution of the MBP to the hybrid product. Details on the construction of the deletion map, the isolation of the *malE–lacZ* fusion strains, and a complete description of the maltose-sensitive phenotype will be presented elsewhere[18,19].

Table 1 Localisation of β-galactosidase activity in strain PB72–47 and representative Mal^R Lac$^+$ derivatives*

Strain	β-galactosidase (Units per mg protein†)	β-galactosidase activity‡ (%)			Response to maltose
		Sonicate	Soluble	Insoluble§	
PB72-47	960	100	23	74	Sensitive
PB72-47-R1	1,483	100	71	25	Resistant
PB72-47-R2	1,275	100	65	33	Resistant

* Mal^R Lac$^+$ mutants were obtained by screening for strains that could form large, blue colonies on maltose minimal agar plates containing the indicator dye 5-bromo-4-chloro-β-D-galactoside.

† All strains were grown to mid-log phase in glycerol minimal medium at 30 C and induced by the addition of 0.2% maltose for the final 180 min of culture. One unit of β-galactosidase activity is that amount of enzyme that will hydrolyse 1 nmol of *o*-nitrophenylgalactoside per min. Note that the original fusion strains themselves are Mal$^-$ due to the inactivation of the *malE* gene. To permit induction by maltose, the hybrid genes were transferred on a λ transducing phage (see Fig. 3) into a *mal*$^+$ genetic background.

‡ Cells were disrupted by sonication[11]. Enzyme activities were normalised with the activity present in the sonicate after the removal of remaining whole cells and are expressed as a percentage of such. Soluble and insoluble refer to the supernatant and pellet, respectively, after centrifugation at 100,000 *g* for 1 h.

§ In a separate experiment, the cytoplasmic and outer membranes were separated according to the method of Osborn *et al.*[17]. >90% of the membrane-associated enzyme activity fractionated with the cytoplasmic membrane.

Selection for export-defective mutants

If the attempt by the cell to export these hybrid proteins is responsible for the maltose-sensitive phenotype, then mutants resistant to maltose should include ones defective in export. Spontaneous mutant derivatives of strain PB72-47 were selected which continue to synthesise the hybrid protein but which are maltose-resistant (Mal^R). Since the most frequent cause of the Mal^R phenotype are mutations eliminating synthesis of the hybrid protein (Mal^R Lac$^-$), only those derivatives which were Mal^R Lac$^+$ were analysed. It was reasoned that such Mal^R Lac$^+$ mutants would no longer attempt to export the protein but, rather, the protein would remain in the cytoplasm. This was so for all the Mal^R Lac$^+$ strains which we obtained (Table 1). The hybrid protein synthesised by several of these Mal^R Lac$^+$ strains seems identical in size to the hybrid protein produced by the parental strain (Fig. 2). However, since these proteins for the most part are no longer membrane-associated, they are apparently subject to proteolytic degradation, most probably at their amino-terminal end, as indicated by the appearance of a second maltose-inducible band. This second band has an apparent molecular weight nearly identical to the monomer subunit of β-galactosidase (116,000). Such degradation has been observed with other hybrid proteins containing a β-galactosidase moiety[13,14].

In strain PB72-47, the amino-terminal extension provided by the MBP moiety of the hybrid protein presumably directs the hybrid protein into the cytoplasmic membrane. One possible explanation for the failure of these Mal^R strains to attempt to export the hybrid protein is that the protein produced by these strains has an altered amino-terminal signal sequence which the cell's export apparatus cannot recognise. If this is so, then the mutation responsible for the Mal^R phenotype must be genetically tightly linked to the hybrid gene. Since the *malE–lacZ* fusion is carried by a λp (plaque-forming) transducing phage, linkage information was obtained simply by isolating the λ phage from each Mal^R Lac$^+$ fusion strain and subsequently using it to lysogenise the *mal*$^+$ Δ*lac* strain MC4100. If the resultant lysogen is Mal^R Lac$^+$, the mutation must have been cotransduced with the hybrid gene. This proved to be so for all of a number of independently-isolated Mal^R Lac$^+$ strains.

Incorporation of signal sequence mutations into the *malE* gene

If the hybrid proteins synthesised by these Mal^R Lac$^+$ strains have an altered signal sequence, the mutations responsible must be located early in the *malE* portion of the hybrid gene. We determined the effect of these mutations on the localisation of

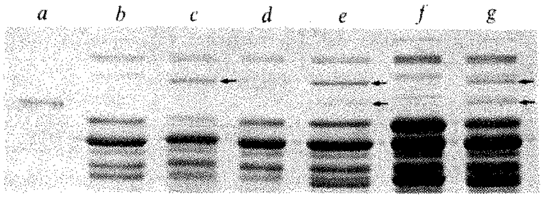

Fig. 2 SDS–polyacrylamide gel electrophoresis of whole cell extracts of *malE–lacZ* fusion strains. Whole cell extracts were prepared and electrophoresis was performed as described elsewhere[20]. Only the relevant upper one-third portion of the gel is shown. *A*, Purified *E. coli* β-galactosidase, 2.5 μg; *b*, strain PB72-47, uninduced; *c*, strain PB72-47, induced; *d*, strain PB72-47-R1, uninduced; *e*, strain PB72-47-R1, induced; *f*, strain PB72-47-R2, uninduced; *g*, strain PB72-47-R2, induced. The hybrid proteins are easily identified as maltose-inducible proteins of high molecular weight (arrows). In addition, Mal^R derivatives exhibit a second maltose-inducible band of molecular weight nearly identical to native *E. coli* β-galactosidase (arrows, see text).

the wild-type *malE* product, the MBP. As described in Fig. 3, these mutations could be recombined onto the wild-type *malE* gene. The resultant recombinants had a Mal$^-$ phenotype when scored on maltose tetrazolium agar, indicating that their ability to utilise maltose as a carbon source had been impaired. However, it was found that these strains would grow slowly on maltose minimal medium. Two of these strains (PB1101 and PB1102), derived from independently-isolated Mal^R Lac$^+$ fusion strains, were characterised further. The mutant alleles were designated *malE101* and *malE102*, respectively.

To confirm that the correct mutations had been incorporated into the *malE* gene by this construction, strains PB1101 and PB1102 were lysogenised by the *L*pp transducing phage carrying the original *malE-lacZ* fusion present in strain PB72-47. The lysogen obtained was maltose-sensitive, but Mal^R Lac$^+$ derivatives were obtained at a very high frequency, a result of the reciprocal recombination event described in Fig. 3. In a similar manner, it was found that the *malE101* and *malE102* mutations could be incorporated by recombination into the hybrid gene of the maltose-sensitive fusion strain PB62-37 (again, resulting in a Mal^R Lac$^+$ phenotype). Since only a small promotor-proximal portion of the *malE* gene is present in this fusion (Fig. 1), this indicates that the *malE101* and *malE102* mutations must, indeed, be located early in the *malE* gene. More precise mapping was complicated by the fact that strains PB1101 and PB1102 can still utilise maltose as a carbon source.

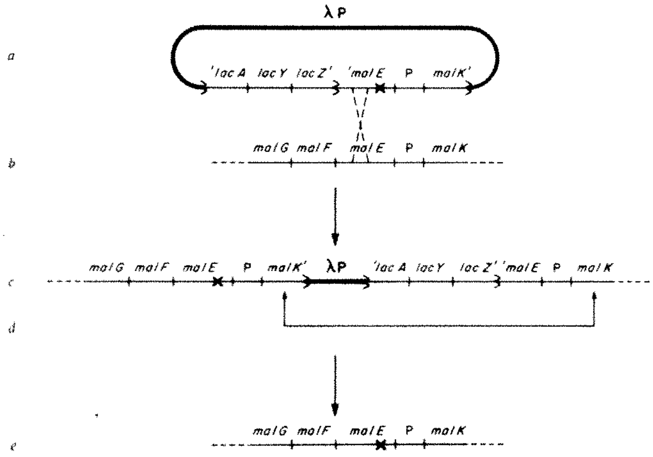

Fig. 3 Recombination of the *malE101* mutation onto a wild-type *malE* gene. *a*, the λp transducing phage that carries the *malE–lacZ* hybrid gene from strain PB72-47-R1. The mutation *malE101* is indicated by an ×. (*b*) A portion of the chromosome of the *mal*$^+$ strain MC4100 which includes a wild-type *malE* gene. The λp transducing phage is Δ*att* and strain MC4100 is Δ*lac*. Thus, lysogenisation can most efficiently be achieved by recombination between their homologous *mal* DNA regions. Integration of the phage DNA as drawn results in a lysogen (*c*) in which the *malE101* mutation has been crossed onto an intact *malE* gene. Curing of the phage DNA by the slightly different homologous crossover indicated by the arrows (*d*) leaves the *malE101* mutation behind in the chromosome (*e*), yielding strain PB1101. Strain PB1102 was constructed in an identical manner.

Identification of a mutant MBP precursor

As it appeared that there might be a defect in the export of the MBP in the mutant strains, cell extracts of strains PB1101 and PB1102 were examined for the form and location of the mutant proteins. Whole cell extracts were prepared from cultures that had been grown on glycerol (uninduced) or maltose (induced) and examined on SDS-polyacrylamide gels (Fig. 4). The maltose-inducible band corresponding to wild-type MBP is not present with either strain. However, a new maltose-inducible band is discerned which migrates with a molecular weight some two to three thousand larger than native MBP. This protein is very similar in size to that reported for the MBP precursor made *in vitro*[9].

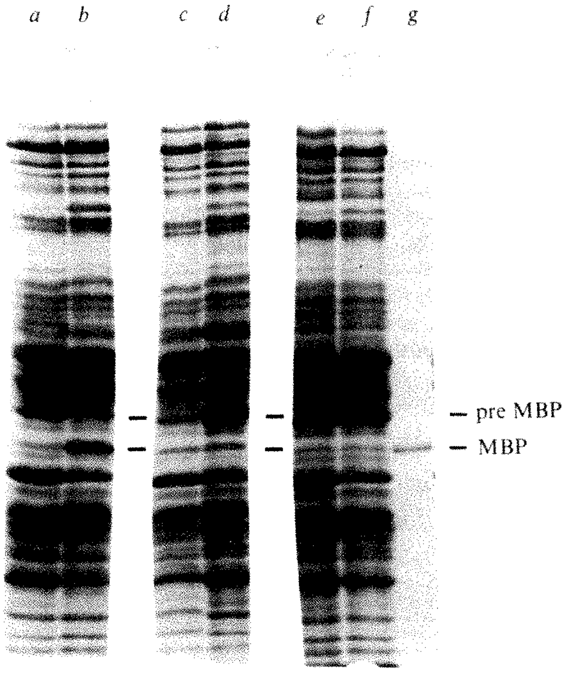

Fig. 4 SDS-polyacrylamide gel electrophoresis of whole cell extracts of *malE* mutant strains. Whole cell extracts were prepared and electrophoresis was performed as described elsewhere[20], except that the acrylamide concentration here is 10%. *a*, Strain MC4100, uninduced; *b*, strain MC4100, induced; *c*, strain PB1101, uninduced; *d*, strain PB1101, induced; *e*, strain PB1102, uninduced; *f*, strain PB1102, induced; *g*, purified *E. coli* native MBP, 2.0 μg. The position of native MBP in the *mal*$^+$ strain MC4100 and the position of the presumed MBP precursor (preMBP) in the two mutant strains are indicated. Everything shown in the figure is from the same slab gel.

The simplest explanation for these results is that strains PB1101 and PB1102 produce a MBP with an altered amino-terminal signal sequence. This sequence is not recognised by the cell's export apparatus and the protein accumulates in the cytoplasm in its larger (unprocessed) form. Different cell fractions of strains PB1101 and PB1102 were prepared and run on SDS gels, in order to determine the cellular location of this new maltose-inducible protein. A maltose-inducible protein which is larger than native MBP is observed in soluble extracts from both strains. This protein is somewhat smaller than the presumed precursor observed in whole cells and is believed to correspond to a breakdown product of the precursor. Only minute amounts of this protein are released by the cell by a cold osmotic shock procedure which is used to operationally define periplasmic proteins in Gram-negative bacteria[15]. Therefore, this protein must be primarily located in the cytoplasm.

In order to verify that the presumed precursors observed in the mutant strains are, indeed, related to the MBP, antiserum prepared against native *E. coli* MBP was used to immune precipitate the soluble extract from a maltose-grown culture of strain PB1101 labelled with ^{14}C-amino acids (Fig. 5). The new maltose-inducible protein observed by gel electrophoresis of the soluble extract of strain PB1101 was specifically precipitated by this antiserum. In addition, several minor proteins in the same molecular weight range were precipitated from the mutant extract. Since none of these proteins was precipitated when the antiserum was pre-incubated with purified, unlabelled MBP, it was concluded that all of the proteins precipitated were antigenically related to MBP. One of the minor proteins so precipitated ran on the gel with an apparent molecular weight identical to native MBP. This may represent a small amount of MBP that was exported to the periplasm in the mutant strain and correctly processed, allowing strain PB1101 to utilise maltose as a carbon source, albeit inefficiently. Additionally, a minor protein so precipitated from the mutant strain ran with the same apparent molecular weight as the presumed precursor observed in whole cells (see Fig. 4). We conclude that this protein is the MBP precursor, and that it is fairly stable in whole cells. However, when the cells are disrupted under non-denaturing conditions, this protein is subjected to some proteolytic degradation, and very little remains intact even though all manipulations are performed at 4°C. Presumably, the enzyme(s) responsible for this proteolytic degradation are located in the periplasm or outer membrane where they are not accessible to the mutant MBP precursor until the cells are broken. However, these do not necessarily have any relationship to the enzyme(s) responsible for processing normal exported proteins to their mature sizes.

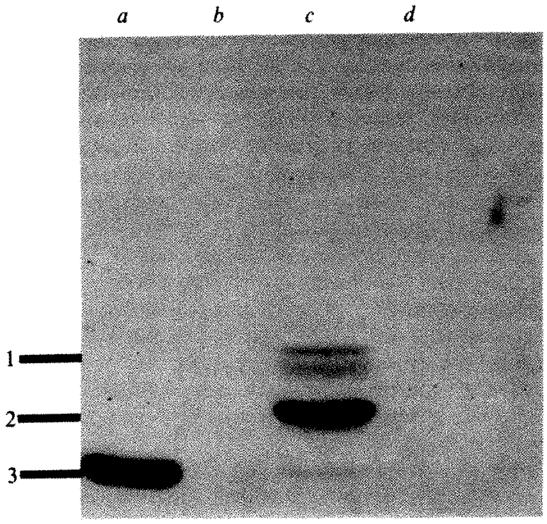

Fig. 5 Immune precipitation of MBP from soluble extracts of strains MC4100 and PB1101. 500 ml cultures were grown in maltose minimal medium to mid-log phase and labelled with ^{14}C-amino acids (50 μCi, NEN) in a 50 ml volume for the final 60 min of culture. Cells were washed twice and resuspended in 5 ml of buffer (10 mM Tris, *p*H 7.2). Cells were then disrupted in a French pressure cell and twice clarified by centrifugation at 100,000 *g* for 1 h. 0.2 ml of the soluble cell extract, 0.1 ml [3 M NaCl, 3% Triton X-100, 10 Mm Tris *p*H 7.2] and 3 μl of rabbit anti-MBP serum (provided by W. Boos) were combined and incubated on ice for 3 h. 30 μl of sheep anti-rabbit IgG (Miles) were added, and the entire mixture was incubated overnight at 4° C. The precipitate obtained was washed three times with 10 mM Tris, *p*H 8.0 (1st wash, 1% Triton X-100; 2nd wash, 0.1 M LiCl; 3rd wash, 0.1% SDS). The final pellet was resuspended in 0.1 ml gel sample buffer, heated at 90 °C for 2 min, and 50 μl were loaded per well for electrophoresis. Finally, the gel was stained with Coomassie blue, dried and autoradiographed. *a*, And *b*, strain MC4100; *c*, and *d*, strain PB1101; *b*, and *d*, anti-MBP serum was pre-incubated for 30 min on ice with 10 μg of unlabelled native *E. coli* MBP. The three lines along the left side of the gel indicate the corresponding positions where the following three proteins are observed to run: (1), The MBP precursor observed in whole cells of strains PB1101 and PB1102. (2), The maltose-inducible protein observed in soluble cell extracts of strains PB1101 and PB1102. And (3), purified native *E. coli* MBP.

Reversion analysis

For both strains PB1101 and PB1102, we obtained *mal*$^+$ revertants by growing these strains in maltose minimal liquid medium through many generations. Since *mal*$^+$ revertants had a considerable selective advantage in such conditions, with time they accumulated to form the majority class of the population. Several independently-isolated revertants from both strains were characterised. In all cases, these appeared to be 'true' revertants, in that the MBP synthesised by these strains is made in normal amounts and found in the periplasm in its native form. That strains PB1101 and PB1102 spontaneously revert to wild-type *mal*$^+$ indicates that the mutations *malE101* and *malE102* result in single amino acid substitutions in the MBP polypeptide.

Discussion

Complete or partial amino acid sequences for the amino-terminal extension of a number of exported proteins have been reported (summarised by Habener *et al.*[16]). Most of these are from eukaryotes. All these sequences contain a central region of some 10 to 12 non-polar and hydrophobic amino acid residues, although it is a different sequence in each case. There is no other feature common to these primary structures which readily identifies itself as being key to its recognition as a signal initiating protein export. We have now isolated *E. coli* strains which fail to secrete the MBP, most probably resulting from mutational alterations in the signal sequence region of this protein. We are now determining the precise nature of the alterations, in order to define that feature of the signal sequence which is essential for initiating export. Confirmation of the location of the mutations in the portion of the *malE* gene corresponding to the signal sequence would provide direct evidence for its role in the initial steps of protein secretion.

Fusion strains have also been used to isolate mutants defective in the localisation of the *lamB* gene product, the phage λ receptor[14]. These mutations most probably result in alterations in the signal sequence of this protein which is normally located in the outer membrane of *E. coli*.

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letters

The discrete source component of galactic γ rays

THE question of the relative contributions from discrete sources and continuum to the flux of cosmic γ rays is a continuing one, with published estimates varying from a 10% source contribution[1] to more than 40%[2] and even to a possible 100%[3]. Since these estimates have mainly been based on parts of the necessarily incomplete set of only 13 known γ-ray sources[4], it is not surprising that such a wide variety of conclusions is possible. The basic reason is the limited angular resolution of contemporary γ-ray detectors ($\simeq 1.5°$), and it is unlikely that great improvements will be made in the near future.

A knowledge of the individual sources of γ rays (and their summed intensity contribution) is relevant to energetic processes in discrete objects, but our own prime interest relates to the fact that useful conclusions about the origin of the particle component of cosmic radiation can be obtained by an analysis of the continuum.[5] The derivation of the magnitude of the continuum after both seen and unseen discrete source contributions have been subtracted off is, therefore, an important task.

Some general guidance might be expected from an analysis of related regions of the electromagnetic spectrum. In X rays, where detectors of high angular resolution are used, the continuum contribution appears to be very small (probably $\lesssim 5\%$); however, that the observed γ rays are not the high energy 'tails' of the X-ray sources is immediately clear from the general lack of correlation of specific γ-ray and X-ray sources.

A comparison with the radio region is probably more relevant. Potential candidates for producing the continuum γ rays are electrons of energy of the order of 1 GeV interacting with the interstellar medium by way of bremsstrahlung and protons of energy 1–10 GeV interacting with the same medium and producing π^0 mesons which in turn decay to γ rays. The relevance of the radio region is that it is generally accepted that the bulk of the radio flux (in the range 10–1,000 MHz) is produced as synchrotron radiation from electrons spiralling in the galactic magnetic field. One GeV electrons produce radiation with frequency 150 MHz and Brindle *et al.*[6] have presented evidence which shows that the continuum radiation provides at least 95% of the flux. Thus, such indirect evidence as there is suggests a predominance of the continuum contribution.

It is apparent from an examination of the longitude distribution of the total γ-ray flux that sources distributed in a similar fashion to main sequence stars are not responsible for much of the flux. The expected large peak towards the galactic centre is absent. Furthermore, the latitude distribution of γ rays is much sharper than that of starlight. Instead, it is likely that the sources have a similar spatial distribution to that of young objects (such as, HII regions), evolved objects (pulsars, supernova remnant) or X-ray sources (the fact that there is not a one-to-one correspondence with known X-ray sources does not preclude this).

We aim here to derive more reliable estimates of the discrete source contribution using both a simple geometrical model and properties of X-ray sources and pulsars as a guide. Since these different approaches lead to very similar results there is some justification for confidence in their validity.

We consider separately the contributions from two possible types of source population: (1) sources with high intrinsic luminosity and low space density (HP sources) and, (2) sources with low intrinsic luminosity and high space density (LP sources).

In the simplest version of the model the sources are distributed uniformly throughout a slab of half thickness z_0. The differential source count frequency from HP sources will then be $N(S) \propto S^{-2}$ for distances r much greater than z_0 and the sources will be closely confined to the galactic plane, while LP sources are numerous enough to give a large number nearer than $\simeq z_0$, these nearby sources being isotropically distributed on the sky. This differentiation of source populations shows very clearly the dependence of the derived limits on the space density of the sources. The use of this double δ-function luminosity function is adequate provided we restrict attention to low latitudes where geometry should influence $N(S)$ more than the luminosity function.

The sources observed by COS B are concentrated within a few degrees of the galactic plane and are therefore of the HP population. For such a population $S_N \propto 1/N$ on average, where S_N is the observed intensity of the N'th brightest source. Using the observed intensities of the N_{obs} brightest sources we can estimate the total intensity of the whole population as

$$J_{\text{T}} = \sum_1^{N_{\text{obs}}} S_{\text{N}} \cdot \sum_1^{N_{\text{max}}} \frac{1}{N} \bigg/ \sum_1^{N_{\text{obs}}} \frac{1}{N} \qquad (1)$$

where $N_{\text{max}} = (r_0/r_1)^2$ is the total number of sources within the distance to the boundary at $r = r_0$ and r_1 is the distance to the nearest source. Equation (1) is intended to minimise the statistical error on the estimate of J_{T} by making use of the summed flux from known sources explicitly. (As written it applies simply to a small range of longitudes over which r_0 can be regarded as constant.)

We apply this result to three COS B observation regions $l = 305°$–$335°$, $60°$–$90°$ and $110°$–$140°$ for which a systematic search for sources has been made[4] and $N_{\text{obs}} = 8$. The anticentre region has been excuded since two-dimensional geometry is probably inappropriate there. Equation (1) then becomes

$$J_{\text{T}} = \text{J}_8 \times 0.37(0.62 + 2\ln(r_0/r_1)) \qquad (2)$$

From ref. 4, $J_8 = 3.8 \times$ Crab flux $= 1.3\ 10^{-5}\ \text{cm}^{-2}\ \text{s}^{-1}$ (>100 MeV) (using the measured Crab flux of $3.5 \times 10^{-6}\ \text{cm}^{-2}\ \text{s}^{-1}$). The main uncertainty is the value of r_0/r_1, although this is not critical because of the logarithmic dependence in equation (2). Since the bright observed sources have latitudes $|b| \simeq 0$–$3°$, the magnitude of r is in the range 1–2 kpc if the thickness $2z_0$ of the galactic source layer is about 200 pc. (a value typical of young objects). The value of r_0 lies in the range 10–20 kpc for the regions considered, so that $J_{\text{T}} = (1.4$–$2.4)J_8 = (1.8$–$3.1)10^{-5}\ \text{cm}^{-2}\ \text{s}^{-1}$ and the average line flux is $(0.9$–$1.5)10^{-5}\ \text{cm}^{-2}\ \text{s}^{-1}\ \text{rad}^{-1}$ (>100 MeV). The average observed total line flux for these regions is $3.8 \times 10^{-5}\ \text{cm}^{-2}\ \text{s}^{-1}\ \text{rad}^{-1}$ (for $|b| < 6°$) so the analysis indicates that between 24% and 40% is from sources.

Source density gradients in the Galaxy may make the use of a uniform two-dimensional geometry inappropriate for the $l =$ 305–335° region because one is here looking in a direction not far from that of the galactic centre; however, for the regions $l = 60°$–$90°$ and $110°$–$140°$, where the gradient is probably small, the average line-flux is $2.8\ 10^{-5}\ \text{cm}^{-2}\ \text{s}^{-1}\ \text{rad}^{-1}$ ($|b| < 6°$)

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Table 1 Alternative estimates of the fraction, f, of γ rays above 100 MeV from discrete sources

Model	Longitude intervals (γ-ray observations)	N	J_T/J_N	$j_S \times 10^5$ cm^{-2} s^{-1} rad^{-1} (>100 MeV)	f
Flat slab	1, 2, 4	8	1.4–2.4	0.9–1.5	0.24–0.40
	1, 2	8	1.4–1.9	0.9–1.2	0.32–0.42
X-ray sources	1, 2, 3, 4	12	1.1	0.9	0.19
Pulsars	1, 2, 3, 4	12	1.05	0.9	0.18

Longitude key: 1, 60°–90°; 2, 110°–140°; 3, 160°–210°; 4, 305°–335°.

and $r_1 \simeq 10$ kpc. Hence $J_T = (1.4–1.9)J_8$ and the discrete source fraction is 32–42%.

A second approach to the HP component follows ref. 3 in assuming that the form of the observed brightness distribution $N(S)$ for X rays is similar to that for γ rays. Since the known sources do not have a constant ratio of γ-ray to X-ray flux, as remarked earlier, we postulate that while γ-ray sources are different objects from X-ray sources, their spatial distribution, luminosity function and total number are approximately the same as for X-ray sources. Since both populations have their brightest sources confined to within a few degrees of the plane a similar spatial distribution at least seems probable. In applying this method we again confine attention to the COS-B observation regions which have been systematically searched for γ-ray sources (the anticentre region can now be included since no particular geometry is assumed). The 72 4U sources[7] in these four areas are found to have a value of J_T/J_N for X-rays which is smaller than for a simple slab model (a consequence of the flattening of the X-ray $N(>S)$ to $S^{-0.4}$ below 200 Uhuru counts). This method gives a slightly lower value for f than the slab model (Table 1). It is interesting to note that if the $N(>S)$ versus S distributions for X-ray and γ-ray sources are normalised at the S intensities for the Crab, then the value of f is virtually the same as found using the method described above.

Although the particular assumptions made in the $N(S)$ method are unlikely to be completely justified the comparison of this result with that from the slab model does indicate that the sensitivity of the estimates to the type of (HP) source population involved is small enough to make the exercise worthwhile.

Another useful deduction from the X-ray data can be made as follows. A prominent feature of the X-ray sources is that there is a much bigger intensity in the general direction of the Galactic Centre than elsewhere. Restricting attention to X rays (and γ rays) within a latitude range of $|b| < 10°$ the ratio of total X-ray intensity in the inner Galaxy to that in the outer is $I_X(270° < l < 90°)/I_X(270° > l > 90°) \simeq 9.4$. This ratio can be compared with that for γ rays above 100 MeV of 2.2. (If the contributions from the Crab (which dominates in the X-ray case) are taken out the ratio is as high as 34 for X rays and only 2.4 for γ rays.) An upper limit can be set as the fraction of the flux of γ rays in the outer direction coming from discrete sources by assuming that the whole of the flux from the inner direction is from such sources. Adopting a ratio of 9.4, which is itself a lower limit, we find $f \simeq 0.25$, that is, in the range $270° > l > 90°$ no more than 25% of the γ-ray intensity is produced by X-ray sources or sources distributed in the Galaxy in a similar fashion to X-ray sources.

For the third estimate of the HP contribution, we have taken the radio fluxes from the Arecibo deep pulsar survey[8], covering $|b| < 4°$, $43° < l < 58°$. This was chosen for its completeness to low radio fluxes, although the region covered is much more restricted than the COS-B observation areas. A similar result to that for X rays is obtained (Table 1). Comparison can be made with our earlier estimate[1] for pulsars ($f = 0.05–0.10$). The fact that the new value is a factor of 2 higher is not surprising since the new result refers to sources in general with an observed intensity distribution similar to that of pulsars whereas the earlier value related to pulsars alone (with a model in which the γ-ray luminosity was proportional to the radio luminosity).

Turning to the LP sources, we estimate the minimum density of such sources which would be required to produce a fraction f of the galactic plane emission without being visible as discrete sources above the current COS B threshold of $S_{\min} \simeq 0.2 \times$ Crab flux. A uniform slab model with thickness $2z_0$ and radial extent r_0 gives a minimum source density

$$\rho = \left\{\frac{1}{2z_0} \cdot f \cdot \frac{j_{\text{obs}}}{S_{\min}} \cdot \frac{1}{[1 + \ln (b_0 r_0/z_0)]}\right\}^3 \left(\frac{\Omega}{3}\right)^3 \tag{3}$$

where Ω is the solid angle of the area of sky considered and j_{obs} is the observed total line-flux. Using $z_0 = 0.1$ kpc, $b_0 = 10°$, $r_0 = 10$ kpc, $\Omega = 1$ sr for the COS B regions $l = 60°–90°$, $110°–140°$ and $305°–335°$ gives

$$\rho \simeq 10^5 f^3 \text{ kpc}^{-3}$$

or a total number of LP sources in the Galaxy of the order of $10^7 f^3$. For $f = 1$ this is about three orders of magnitude lower than that of main sequence stars, but two orders of magnitude higher than that of likely sources such as pulsars[9] and a possible population of weak X-ray sources[10] (up to 10^5 sources with X-ray luminosity 10^{32} erg s^{-1}). Alternatively, we can state that a maximum of about $100^{-1/3}$, that is, 20%, of the galactic emission could originate in sources of this latter density.

In conclusion, Table 1 shows that the most likely value of f is $\simeq 0.25$ although it could be as high as 0.4 (and not inconsistent with the estimate of Bignami *et al.*[2]). Such a value would not invalidate the interpretation of γ-ray data in terms of a gradient of cosmic rays in the Galaxy, at least in the outer Galaxy[5]. A final remark is that the so-called discrete sources of γ-rays will include complexes of molecular clouds which provide target nuclei for cosmic rays; a derivation of their contribution must await more comprehensive surveys of such cloud complexes.

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Creation of magnetic monopoles in pulsars

THE symmetry of the free Maxwell equations under transformation of electric field into magnetic field led Dirac[1] to suggest the existence of magnetic monopoles. Many experimental searches for these particles have been made on different accelerators and using cosmic ray interactions; no magnetic monopoles having been detected, upper limits of the cross sections for the creation of monopoles have been obtained. The data of Ross *et al.*[2] are the best and can be fitted by:

$$\sigma_{\text{pn}} \leqslant 5 \times 10^{-44}\, m_g^3 \tag{1}$$

where σ_{pn} is the proton–nucleon cross-sections in cm^2, and m_g

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the monopole mass in GeV c^{-2}. It is believed[3,4] that very energetic particles are created in pulsars. These particles can interact between themselves and/or be smashed onto the neutron star surface to produce pairs of magnetic monopoles and antimonopoles. These monopoles (if they exist or are effectively produced) would be accelerated in the pulsar magnetic field, one species towards the interstellar medium, the other towards the neutron star, producing a decrease in the magnetic field. Such a phenomenon can be related to observations, particularly to the discrepancy between the characteristic and the kinematical ages. We show here that these observations can be explained by a production of magnetic monopoles, inherent to the pulsars, with a cross-section lower than equation (1), (that is, without contraction with the negative results of the terrestrial searches). More specifically, we will consider the magnetic monopoles production on the neutron star surface, in the Ruderman and Sutherland model[4]. Other models (with and without gaps) will be considered elsewhere.

Ruderman and Sutherland[4] argue that the creation of polar gaps should take place in pulsars. These polar gaps would be broken by electron pair creation and the subsequent avalanche development, with the potential across the gap

$$V = 1.6\times10^{12}B_{12}^{-1/7}T^{-1/7}\rho_6^{4/7} \tag{2}$$

where $\rho = 10^6\rho_6$ is the curvature of the magnetic field lines at the neutron star surface in cm. The creation of magnetic monopoles is then possible due to the electrons accelerated by the potential of equation (2) across the gap and which crash on the neutron star crust.

The energy threshold E_{eth} for creation of monopoles with the mass m_g on the crust nucleons of mass m_n is

$$E_{\mathrm{eth}} \approx 2m_g^2 m_n^{-1} \tag{3}$$

With the energy eV given by equation (2), the electrons have enough energy to create monopoles with $m_g \leqslant 30$ GeV c^{-2}. Inside the neutron star crust, the electrons lose their energy due to bremsstrahlung and the number of nucleons seen by the electrons before they are slowed down below the threshold energy for monopole creation is

$$\kappa_e \approx \beta X_0 \approx 8.4\times10^{24}\,\beta \quad \text{nucleons cm}^{-2} \tag{4}$$

where X_0 is the radiation length and $\beta = \log(\mathrm{eV}/E_{\mathrm{eth}}) \approx 1$ practically independent upon the pulsar properties. The flux of the magnetic monopoles pairs produced in one polar gap is

$$j_g = \kappa_e \pi r_p^2 nc\sigma_{\mathrm{en}} \approx 6.3\times10^{54}\, B_{12}R_6^3T^{-2}\sigma_{\mathrm{en}} \text{ monopoles s}^{-1} \tag{5}$$

where σ_{en} is the cross-section for the monopole creation in the electron–nucleon interactions. The radius of polar gap r_p and the density of created e^+e^- pairs, n, are given in ref. 4.

Kalman and Ter Haar[5] pointed out that the magnetic monopoles are accelerated by the pulsar magnetic field up to 10^{18} eV and interacting with neutron star crust should produce a cascade of 'secondary' monopoles. They estimate the number of secondary monopoles produced by a primary monopole to be

$$N^* = 6\times10^7\, B_{12}^{1/4}\rho_6^{1/2}n_g^{-1/4}m_g^{-1} \tag{6}$$

where n_g is the magnetic monopole charge in units of 137 e. Half of the monopoles which could be produced in this model are accelerated towards the neutron star surface and with the multiplicity this gives a decrease in the pulsar magnetic field at a rate

$$\frac{\mathrm{d}B}{\mathrm{d}t} = -\frac{j_g N^*}{4}\frac{137\, n_g e}{R^2} \tag{7}$$

It is commonly assumed that the slowing down of the pulsar results from the loss of rotational energy by magnetic dipole radiation. This allows us to estimate the neutron star magnetic field as a function of the period T and of its derivative $\dot{T}$

$$B \approx 8.3\times10^{18} I_{44}^{1/2}R_6^{-3}(T\dot{T})^{1/2} \quad \text{G} \tag{8}$$

where $I = 10^{44}\, I_{44}$ is the moment of inertia of the neutron star in g cm^2 and $R = 10^6R_6$ is its radius in cm. Equation (7) can then be integrated and the magnetic field expressed as a function of the period T:

$$B(T) = B_i\left\{1 - \frac{\sigma_{\mathrm{en}}}{\sigma_{\mathrm{crit}}}\log\left(\frac{T}{T_i}\right)\right\}^{4/7} \tag{9}$$

with

$$\sigma_{\mathrm{crit}} = 6\times10^{-46}\frac{R_6^5B_{i12}^{7/4}}{I_{44}\rho_6^{1/2}}\frac{m_g}{n_g^{3/4}} \quad \text{cm}^2$$

where T_i and B_i are the period and the magnetic field at the creation of the gap ($\approx$ the creation of the pulsar).

There are observations to test this decrease in the magnetic field. First, it implies that the characteristic ages $\tau = T/2\dot{T}$ are not true indicators of the real ages but must be greater. Actually there is a discrepancy between the kinematical ages τ_k (obtained from the direct measurements of the proper motion and the distance from the galactic plane of the pulsars) and the characteristic ages, which seem greater[7]. For those pulsars whose proper motion was measured, the mean kinematical age is $\langle\tau_k\rangle = 1.3\times10^6$ yr while the mean characteristic age is $\langle\tau\rangle = 5\times10^6$ yr, and if the same mean velocity is used for all the pulsars a much larger discrepancy is found. Furthermore, some pulsars are remarkable because of their extraordinarily great characteristic ages—up to 10^9 yr—a fact which could very well be explained by a decrease in the magnetic field, too. Considering equation (9), it can be seen that the creation of magnetic monopoles in pulsars may explain these observations if $\sigma_{\mathrm{en}} \leqslant \sigma_{\mathrm{crit}}$. When this cross-section is compared with the upper limit of equation (1) from the monopole searches using cosmic rays, it can be seen that there is no contradiction with this explanation and the fact that monopoles are not observed directly: expecting that the alpha-numeric coefficient $R_6^5B_{i12}^{7/4}/I_{44}\rho_6^{1/2}$ to be of the order of unity, we have for the monopole production cross-section in pulsars:

$$\sigma_{\mathrm{en}} \leqslant 6\times10^{-46}\frac{m_g}{n_g^{3/4}} = 3\times10^{-44}\ \text{cm}^2$$

$$\text{for } m_g = 30 \text{ GeV } c^{-2} \text{ and } n_g = 0.5 \tag{10}$$

which is considerably smaller than the terrestrial upper limit of equation (1):

$$\sigma_{\mathrm{pn}} \leqslant 10^{-39}\ \text{cm}^2 \quad \text{for} \quad m_g = 30 \text{ GeV } c^{-2} \tag{11}$$

(Besides, the interactions for the production of monopoles in pulsars involve high energy electrons, while the upper limits published so far concern nucleons.) Therefore, as an explanation of the discrepancy between τ and τ_k and of the very great characteristic ages, we suggest that this comes from a decrease in magnetic field due to monopole creation in pulsars with the cross-section of equation (10). This provides an indication of the existence of the magnetic monopoles and of their creation in pulsars or at least (if another explanation for the observations is preferred) a new upper limit on the monopole production cross-section, far more stringent than the terrestrial ones.

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Possible nuclear origin of Ap and Am stars

MANY possible mechanisms have been proposed to explain the abundance anomaly of heavy elements on Ap and Am stars. Van den Heuvel[1] suggested that these stars had been secondaries of binary systems, whose primaries exploded as supernovae and caused the accretion of heavy elements on the secondaries. The abundance distribution of elements with $Z \geqslant 40$ in those stars is generally similar to that of r nuclei in the Solar System, although the overabundance factor for each element relative to Solar System r abundance is not constant, being exceptionally high in some limited regions such as the region around Te, the rare earth region around Nd and Sm, and around Pt and Hg. These observational trends suggest that the abundance pattern in those stars may be a superposition of some specific elements with anomalously high abundance on a background proportional to the Solar System r abundance. Here I suggest an alternative possibility for the nuclear origin of anomalous abundance in Ap and Am stars.

In the early expanding period of supernova shells, the Rayleigh–Taylor instability[2], plasma instabilities or, at a later period, some instability accompanying dust formation[3] would occur. These instabilities might cause more or less pronounced density and composition inhomogeneities and chemically inhomogeneous clumps or knots of clouds might appear in the expanding shells. The clumps would have envelopes surrounding them[2], so that the movement of these clumps would generate turbulence in the expanding shells and tend to chemically homogenise the supernova shells. We can therefore imagine that in the expanding supernova shells there are chemically inhomogeneous clumps embedded in and co-expanding with the chemically homogeneous background gas. These clumps would appear $\leqslant 4 \times 10^{14}$ cm from the supernova[2]. Taking the distance between the components of a typical binary system to be $\sim 10^2$ AU, the instabilities in the shells would occur during the transfer towards the secondary, and the accreting matter on the secondary would probably be composed of some clumps and the background gas, now partially chemically homogenised.

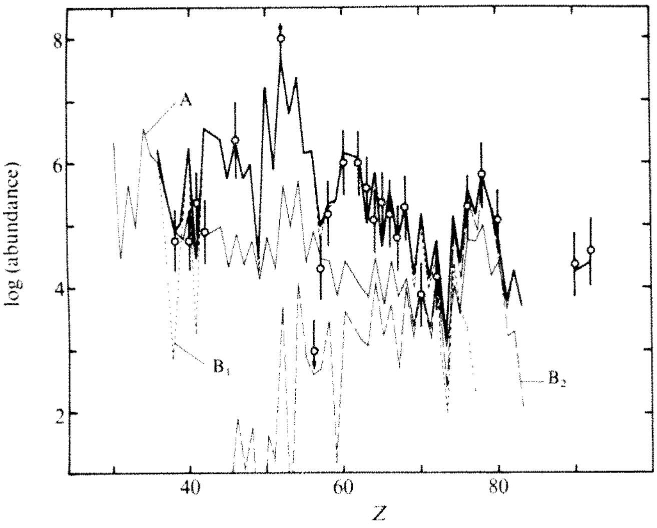

Fig. 1 Abundances of heavy elements on HR465 in the scale $H = 10^{12}$. Open circles with error bars show observations, whereas the heavy solid lines denote the calculated abundances. Arrows indicate the data with only upper or lower limits. Observational uncertainties are unclear but are assumed here arbitrarily to be ±dxp(0.5), although they could be higher. The calculated abundances are composed of the background (A) and two components B_1 and B_2 intrinsic for HR465. They are shown by light solid, short dashed and long dashed lines, respectively. Abundance patterns for the component A in the region of $Z \geqslant 90$, B_1 in $42 \leqslant Z \leqslant 68$ and B_2 in $74 \leqslant Z \leqslant 80$ are substantially the same as those given by the heavy solid lines.

If we restrict the discussion to the region $Z \geqslant 40$, the nuclei formed in the supernova shells are only r and p nuclei. Using Arnett's model[4] of the presupernova with $M_\alpha = 8M_\odot$, I have investigated the nuclear synthesis occurring in the innermost shell of exploding supernovae[5]. The r process is found to take place when the star is within the lagrangian mass range $M_r = 1.34\text{–}1.69 \times 10^{33}$ g. The number of free neutrons per seed nucleus gradually increases with the decrease of M_r in that lagrangian mass range, and therefore the appearance of the abundance curve of produced r nuclei varies with position in the r shell. The shell-intergrated abundance over the r shell, however, can well reproduce the relative abundances of r nuclides in the Solar System. If the r nuclei are produced in the above lagrangian mass range, they become overabundant in the supernova shells as a whole by a factor of $\sim 3 \times 10^4$ as compared to the Solar System r abundance, whereas the overproduction factor for the p nuclei is 10^{0-3} only in the p nuclei-producing region[6]. Hence in the region $Z \geqslant 40$ the abundance distribution in the background gas which accretes on the secondary star would be substantially that of the r process. However, some accreting clumps with a high abundance of r elements might be produced at some position in the r shell. Where these clumps accrete, chemically inhomogeneous spots with intrinsic distribution of r elements would form. It would therefore be possible to observe the abundance distribution due to these spots, being superimposed on the background distribution. The extent of the strong overabundance in some specific elemental regions depends on the mass of accreting clumps relative to the mass of accreting homogeneous gas.

Cowley[7] points out that in the rare earth region the abundance of individual elements in Ap and Am stars can be expressed as two components—the ordinary abundance pattern with a normal odd-even effect (component A), and an abundance distribution with a maximum centred around intermediate rare earths (component B). According to my proposed model, these components A and B correspond, respectively, to the background with the r abundance appearance and to the intrinsic component originating from a chemically inhomogeneous spot.

As an example, consider HR465 observed in 1960 at its rare earth maximum. Figure 1 shows the observations[8–10] in the region of $Z \geqslant 40$ together with the theoretical abundances which consist of the 'background' (this is proportional to the r abundance observed in the Solar System[5,11]: component A) and two components B_1 and B_2 intrinsic for HR465. The components $B_{1,2}$ have the r abundances produced in the regions around $M_r = 1.48$ and 1.37×10^{33} g, respectively. The calculated abundances have been obtained by the superposition of three components so as to minimise the deviation from observations. The relative numbers of nuclei with $Z \geqslant 36$ are

$$A : B_1 : B_2 = 1.0 : 35.5 : 0.45$$

The calculation can well explain the observed data within the observational uncertainties. The component B_1 makes the regions around $Z = 42$, 52 and 60 overabundant, while component B_2 has a maximum centred around Pt. The abundances observed in the other Ap and Am stars are also explained by superimposing some intrinsic abundance patterns on the 'background'. We also expect to observe stars which do not require an additional intrinsic component, and which are explained by the 'background' component. Stars such as 32 Aqr, 63 Tau and γ Equ = HD201601 might be of this type.

Figure 2 shows the mass fraction of the region where a given element is produced in excess to the total mass of the r shell. A given element is said to be in excess at some position i in the r shell when the quantity $N(Z)_i - \langle N(Z) \rangle$ becomes positive, where $N(Z)_i$ is the number of nuclei with Z per unit mass produced at i and $\langle N(Z) \rangle$ is the average value over the entire r shell. Figure 2 implies that, if the sites for Rayleigh–Taylor

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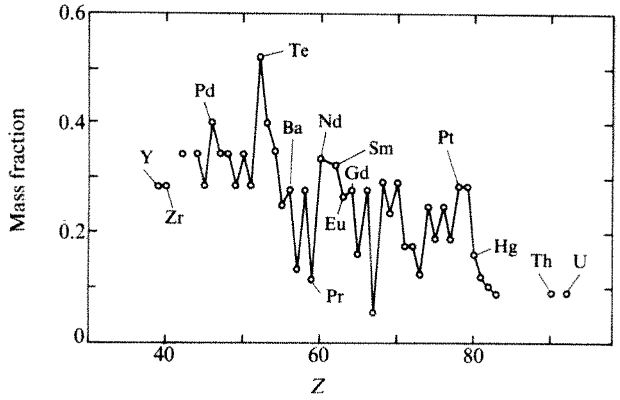

Fig. 2 Fraction of the mass of the region where a given element is produced in excess relative to the total mass of the r shell. Atomic symbols are given for representative elements frequently observed in Ap and Am stars.

instability or plasma instability are randomly distributed in the r shell, the probability that elements near $Z = 52$ will appear in Ap and Am stars with exceptionally high overabundance factors compared to the other elements is by about twofold higher than the probabilities for the other elements. Furthermore, in the light rare earth region the probability is slightly higher than that for heavy rare earths. The occurrence of Ap and Am stars with a high overabundance factor for a given element would show a pattern similar to that in Fig. 2 provided my hypothesis is the only mechanism operative.

Although the hypothesis well accounts for the gross feature of abundance curves on the Ap and Am stars, it cannot always explain the details of the curves; for example, it fails to explain the low abundances of Ba, La and Yb in HR465. For a complete and detailed explanation, secondary processes such as the magnetic accretion or the diffusion process would have to be introduced.

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Consequences of the day-to-night variation of the exospheric temperature of Venus

DURING the exploration of the planet Venus by the Soviet space probe Venera 9, we measured the quantity of hydrogen in the upper atmosphere and its temperature through the optical observation of far UV atmospheric emission. The temperature at the altitude of 250 km (the upper boundary of the atmosphere) was found to vary from 500 K on the dayside to 200 K on the nightside. This variation is compatible with our current knowledge of the heating of upper atmosphere by the solar radiation. It is shown here that this variation, which is larger than on Earth, induces an exospheric transport (out of the atmosphere) of hydrogen from the dayside to the nightside. As an indirect consequence, this transport allows the existence of a large quantity of atomic oxygen in the upper atmosphere, a feature which was still controversial.

Atomic hydrogen in the upper atmospheres of planets is a good tracer of the exospheric temperature[1]. As it is produced through photochemistry of hydrogen compounds in the chemosphere, its distribution and total quantity are connected mainly to the mixing ratio of hydrogen compounds and to the efficiency of vertical transport[2,3]. As hydrogen atoms are scattering Lyα solar photons, they can be easily observed optically, from outside the atmosphere of the planet, through their Lyα emission at 1,215.6 Å.

The Lyα photometer which was flown in October 1975 in the orbiter part of Venera-9 around Venus, allowed intensity and line shape measurements to be made, which have been described previously[4]. The detector was a NO counter used in a photon counting mode, measuring the Lyα emission I_0 through a hydrogen absorption cell in a field of view of 20′. When the cell is activated at an optical thickness $\tau = 10 \pm 1$ (with the help of a tungsten filament which dissociates H_2 molecules into atoms), a part of the Lyα emission line is absorbed in the cell, and the intensity measured by the NO counter drops to $I_\tau = RI_0$. The reduction factor R is a function of the emission line profile and of the Doppler shift of the spacecraft along the line of sight[5].

Our previous report[4] was devoted to the analysis of the data obtained on the sunlit part of the atmosphere above the planetary disk. A hot component was found to be present about 3,000 km of altitude, and associated with the possible effect of the solar wind on the hydrogen exosphere, when it becomes turbulent after bow shock crossing. Below 3,000 km of altitude, the exospheric temperature T_c was found to vary on the dayside from one day to the other between 400 and 600 K, with a mean value of 500 K, associated with a density of 1.5×10^4 atom cm^{-3} at 250 km, the exobase level.

This report analyses the measurements of intensity and reduction factor obtained for lines of sight intersecting the planetary disk, both in the dark and bright parts. These measurements are compared with model predictions of intensity and linewidth, built from a vertical distribution of hydrogen and

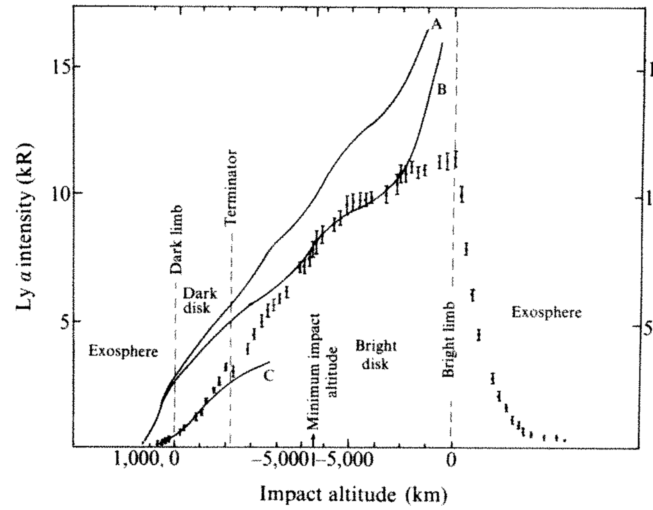

Fig. 1 The reduction factor (which measures the effect of the absorption cell on the Lyα signal when it is activated) is plotted as a function of impact altitude of the line of sight, counted negatively when the line of sight intersects the planetary disk. Theoretical curves are shown for various exospheric temperatures for comparison. The general decrease from left to right (increase of cell absorption) is due to decreasing Doppler shift. On the bright disk, the difference between measured and computed is attributed to contamination by O(I) 1,304 Å line. The observed temperature increase in the exosphere is attributed to bow shock interaction of decelerated solar wind protons with the hydrogen corona[4].

the solution of the radiative transfer equation in the approximation of a spherically symmetric exosphere[6]. The line profile is a by-product of the radiative transfer solution[7]; a theoretical value of the reduction factor R can then be computed, as the Doppler shift is known.

There is some contribution of the large emission of O(I) at 1,304 Å to our measured intensity signal over the bright disk data, as in the case of the Earth, even if our NO counter is less sensitive by a factor of $\simeq 3$ at 1,304 Å than at Lyα. We have used the hydrogen cell measurements for estimating the stray contribution of O(I) as follows.

The values of R measured over the disk are compared to the theoretical values computed for three hydrogen vertical distribution: same vertical distribution below 250 km with density at 250 km $n_c = 1.5 \times 10^4$ atom cm^{-3}, but three different exospheric temperature $T_c = 400$, 600, 800 K (Fig. 1). All curves, experimental and theoretical, decrease from left to right when the line of sight of the instrument scans the planet because during the same time, the Doppler shift due to the orbital motion decreases from -7.7 to -2.6 km s^{-1} and therefore the absorption peak in the cell moves towards the centre of the Venus emission line. When the Doppler shift is equal to the half-width of the absorption profile, one half of the emission line is absorbed and the other is not, whatever the line profile (within limits relevant to the present discussion), and therefore at that point all theoretical curves intersect at a value of $R = 0.5$ (ref. 8). The fact that, for this particular value, the measured R is >0.5 indicates the presence of a non absorbed signal which can be identified with the O(I) contribution to our signal and provides an estimate of its value. On all six orbits analysed, a subtraction of 23% of the bright disk intensities does bring back the measured values of R among the theoretical curves; this corresponds to a 0.8 ratio of the intensities $I(\mathrm{O(I)})/I(\mathrm{H})$, in fair agreement with previous measurements[9].

After subtraction of O(I) contamination (on the bright disk only), the intensity measurements were compared to theoretical intensities (Fig. 2) corresponding to two distributions of hydrogen. Both have the same exospheric distribution determined by $n_c = 1.5 \times 10^4$ at cm^{-3} at 250 km and $T_c = 500$ K (parameter deduced for the daylight exosphere from our previous analysis[4]). Below 250 km, the density follows the Liu and Donahue[10] model distribution with the following cloud top volume mixing ratios: $f_{\mathrm{HCl}} = 6 \times 10^{-7}$, $f_{\mathrm{H_2}} = 7 \times 10^{-8}$, $f_{\mathrm{H_2O}} = 3 \times 10^{-6}$ for model B and $f_{\mathrm{H_2O}} = 2.5 \times 10^{-6}$ for model A. Both models have the eddy coefficient K taken at 10^8 cm^2 s^{-1} and the limit of complete absorption of Lyα by CO_2 is set at 110 km. Differences between numerical values used by Liu and Donahue and us are of minor importance: we took, as our measurements indicated we should, $T_c = 500$ K instead of 350 K, and $n_c = 1.5 \times 10^4$ cm^{-3} at 250 km instead of $n_c = 10^5$ at 210 km. This last point corresponds to scaling down the Liu and Donahue density distribution by a factor of 5. According to the analysis of Sze and McElroy[11], this should imply approximately a decrease of hydrogen compounds at the cloud top level by the same factor of 5.

The results of the comparison are: (1) On the dark side, the theoretical intensities are nearly insensitive to hydrogen content below 250 km, region not illuminated: the main contribution comes from the part of the line of sight directly illuminated. Owing to the orbital configuration, this region of emission covers the twilight region behind the terminator, from 90° to roughly 120° of solar zenith angle. The measured intensities are much smaller than on either model, indicating that on the nightside of Venus the exosphere contains much less hydrogen than on the dayside. As shown on Fig. 2, a good fit is obtained with model C, identical to B below 250 km but with a lower temperature $T = 200$ K. Even lower temperatures would be required, if a density at exobase level was taken larger on the nightside than on the dayside, by analogy with the case of the Earth where the night-to-day ratio of hydrogen densities at the exobase level is of the order of 2, with a day-to-night temperature ratio of 1.3. We conclude that a large difference of exosphere temperature between day and night is demonstrated on Venus. A similar conclusion was reached from Mariner 5 results[12], but with a more complicated analysis restricted to limb measurements. Such a large day-to-night temperature ratio is indeed predicted from an elaborate theoretical model[13] of the upper atmosphere of Venus, which includes as a parameter the degree of efficiency of solar heating. Our dayside temperature of 500 K would correspond in this model to a medium efficiency solar heating.

(2) On the bright disk, the intensity is very sensitive to the hydrogen content below 250 km. A good fit is obtained with model B except near the bright limb, where field of view effects may smooth out the large theoretical increase. A Liu and Donahue model scaled down to our values gives a satisfactory description of the situation. We therefore have to discuss their conclusions. With their mixing ratio of hydrogenous compounds at cloud top (total hydrogen mixing ratio at least 10^{-6}) the production of hydrogen is large and because there are only three sinks (Jeans escape, non thermal escape, downward transport and recombination described by the eddy coefficient K) and as the Jeans escape is small, either the non thermal escape flux ϕ_{nt} or the eddy coefficient K must be very large. This places constraints over oxygen. If K is large, oxygen atoms are removed by transport and the dayglow at 1,304 Å is not due to optical resonance but to electron excitation. If K is small, oxygen atoms exist in large quantities and the dayglow is due to optical resonance, but in this case a very large non thermal escape flux is needed. The modifications our data force on the Liu and Donahue analysis ($T_c = 500$ K instead of 350 K, n_{250} scaled down by a factor of 5 which implies a value of $f_{\mathrm{H_2O}}$ around 0.5×10^{-6} at cloud top) do not change their conclusion. But what may change the discussion is the large asymmetry between day and night that we have demonstrated. On Earth, the density ratio adjusts itself to the temperature ratio in such a way that, at each point of the exobase, the upward flux of ballistic hydrogen atoms (a function of local conditions) is approximately equal to the downward flux of ballistic hydrogen atoms which comes from everywhere on the exobase. This seems not to be the case on Venus: the large difference in temperature between day and night, which results probably from the slow rotation of the planet, implies a large transport of hydrogen from the dayside to the nightside (or lateral flow) and the net upward ballistic flux

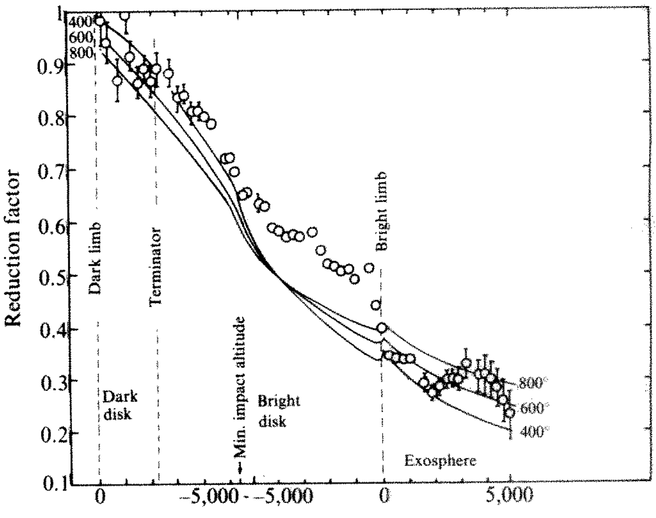

Fig. 2 The measured Lyα intensity (after subtraction of 1,304 Å contribution on the bright disk) is compared with computed intensities, for three different models A, B, C of hydrogen vertical distribution. A and B are different only below the exobase ($T_c = 500$ K) which shows that bright disk intensity is sensitive to hydrogen below the exobase. B and C are different only above the exobase, where model C is at $T_c = 200$ K. Dark disk intensity is sensitive to the hydrogen above the exosphere, directly illuminated by the Sun. $n_c = 1.5 \times 10^4$ atom cm^{-3}.

can become positive. For a sinusoidal variation of the temperature between $T_{\max} = 500$ K at the subsolar point and $T_{\min} = 200$ K at the opposite point, and a uniform density distribution $n_c = 1.5 \times 10^4$, we computed this net upward ballistic flux to be 5×10^7 atom cm^{-2} s^{-1} for the subsolar point. This upwards flux could very well play the role of the nonthermal escape flux ϕ_{nt} introduced by Liu and Donahue[10] and Sze and McElroy[11]. When reported on their Figs 2, 3, 4, $\phi_{nt} = 5 \times 10^7$ cm^{-2} s^{-1} implies low values of K of the order of 10^6 cm^2 s^{-1}. This value could, therefore, allow the atomic oxygen to build up and the 1,304 Å emission would be explained by resonance scattering.

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Direct experimental verification of the Kelvin equation for capillary condensation

THE thermodynamic properties of liquids trapped in microscopic pores or existing as very small, highly curved droplets are described by the Kelvin equation[1]. This equation forms the basis of critical nucleation theory[2] and has been used in interpreting such diverse phenomena as adhesion[3], the enhanced solubility of small particles[2] and the retention and flow of liquids in porous materials[4–6]. The validity of the application of the Kelvin equation to such highly curved interfaces (where the mean radius of curvature can be in the range 1–100 nm) has been questioned[1,4], but has never been tested by direct experiment. We have used multiple beam interferometry to observe the formation of capillary condensed liquid between crossed cylinders of molecularly smooth mica. We report here that the Kelvin equation is obeyed by cyclohexane menisci with mean radius of curvature as low as 4 nm. We further conclude that the application of the laws of thermodynamics, and the concept of a bulk surface tension, are valid in principle for such highly curved interfaces.

The Kelvin equation[1] (equation (1)) gives the equilibrium mean radius of curvature r_m of a liquid–vapour interface in terms of the actual vapour pressure p and the saturated vapour pressure p_s. The equation can be applied to both liquid drops (r_m positive) and to bubbles and capillary-held wetting liquids (r_m negative)[2]. We have measured the mean radius of curvature of cyclohexane menisci at the periphery of liquid bridges trapped between two crossed cylindrical mica surfaces (Fig. 1) at equilibrium with relative vapour pressures in the range $0.71 < (p/p_s) < 0.94$, giving mean radii of curvature in the range $4\ \text{nm} < -r_m < 19$ nm. The experiments were carried out using an apparatus[7] in which the distance D between the molecularly smooth surfaces of a pair of rigidly mounted crossed cylindrical mica sheets was controlled and measured to ±0.1 nm. Distances were measured by multiple beam interferometry, which also

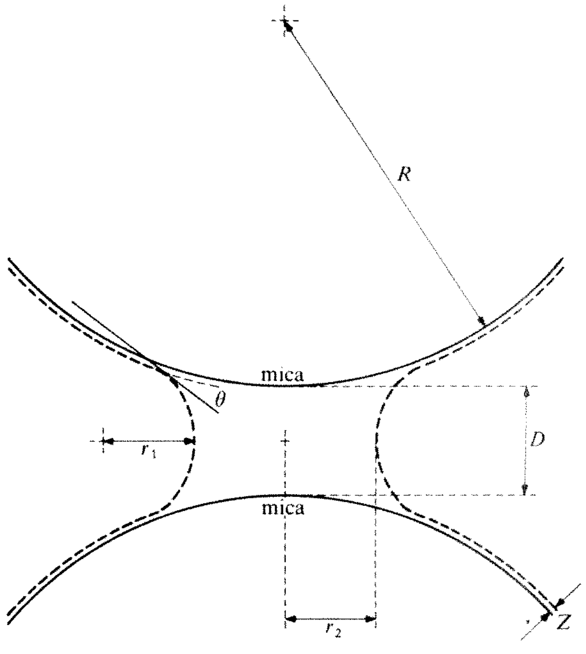

Fig. 1 Space between crossed mica cylinders with bridging meniscus (section through 45° plane of symmetry). Not drawn to scale: $R \approx 10^7$ nm, $r_2 \approx 10^4$ nm, $r_1 \approx -10$ nm. The mean radius of curvature r_m is defined as $1/r_m = 1/r_1 + 1/r_2$. By convention, r_1 is negative and r_2 positive in the configuration shown here. The equation used to calculate values of r_m from experimental values of the critical distance $D = D_c$ (see text) was $r_m \approx r_1 = -(D_c - 2Z)/2 \cos\theta$. This equation is accurate to within 0.5% for the range of values of r_m examined. The measured value of the contact angle θ was $\theta = (6 \pm 1)°$ (L.R. F., to be published).

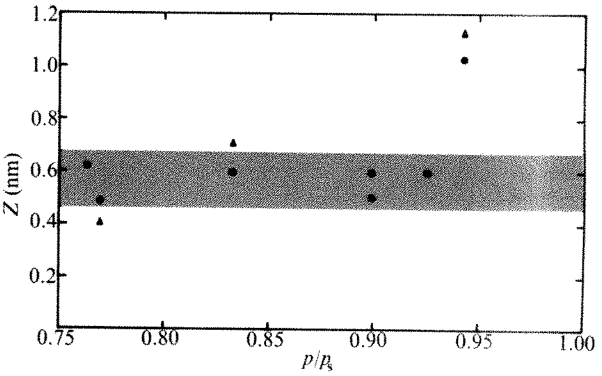

Fig. 2 Adsorption isotherm of cyclohexane on cleaved mica. Values of the adsorbed film thickness Z were determined (1) by measuring the distance (D_{contact}) between the mica surfaces at the moment of first contact, and assuming that $Z = D_{\text{contact}}/2$; or (2) by calculation from the measured root mean square refractive index (μ_{rms}) of the medium between the surfaces when the surfaces were held at a constant small distance (D) apart[8]. △, Points calculated from spacing at contact; ○, points calculated from r.m.s. refractive index. Shaded band: range of possible values of the thickness of a cyclohexane monolayer, calculated from measurements on a Courtauld spacefilling molecular model. We have taken $Z = (0.6 \pm 0.1)$nm for relative pressures below 0.93 and $Z = (1.05 \pm 0.1)$nm for $p/p_s = 0.943$.

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permitted observation of the presence of a bridging meniscus and measurement of the refractive index of the material between the surfaces[8]. For distances D greater than a critical distance D_c, the liquid bridge evaporated irreversibly. Values of r_m were calculated from the experimental values of D_c, with allowance by simple subtraction for the thickness Z of the adsorbed film. Values of Z were determined from the adsorption isotherm at 293 K (Fig. 2).

The Kelvin relation between r_m and (p/p_s) is[9]:

$$\ln (p/p_s) = \{M/\rho_l RT\}\{(\gamma/r_m) - (p - p_s)\} \qquad (1)$$

where M is the molecular weight, ρ_l is the liquid density, p/p_s is the relative vapour pressure, γ is the liquid–vapour interfacial tension at the absolute temperature T, and R is the gas constant. As $|\gamma/r_m| \gg |(p - p_s)|$ for the range of values of r_m measured, this equation gives an almost linear plot of $\ln (p/p_s)$ versus $1/r_m$, with a slope of $[\gamma M/\rho_l RT]$. Two theoretical plots of $\ln (p/p_s)$ versus $1/r_m$ are shown in Fig. 3, together with the values of $1/r_m$ calculated from the experimental values of Z, D_c and θ at various experimental values of $\ln (p/p_s)$. The experimental points agree closely with those predicted by the Kelvin equation, assuming γ = constant, down to the smallest mean radius of curvature examined ($r_m = -4.2$ nm). If allowance is made for the effect of curvature on surface tension[10], a theoretical line is obtained with a slightly better fit to the experimental points. The assumption that an equilibrium adsorbed monolayer affects only the geometry of the system and not the meniscus curvature seems to be justified, although this assumption may need modification for thicker adsorbed layers[11].

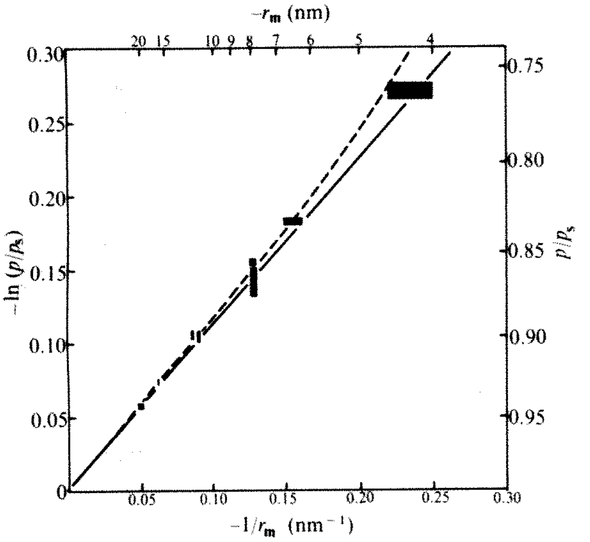

Fig. 3 Comparison of theoretical (Kelvin equation) plot of $-\ln (p/p_s)$ versus $-1/r_m$ with experimental results. The solid line corresponds to the assumption that γ ($= 25.5$ mN m^{-1}) is independent of r_m. The dashed line is calculated assuming the approximate relation[10] $\gamma = \gamma_\infty(1 + \Delta\lambda/r_m)$, where γ_∞ is the surface tension of a planar cyclohexane liquid–vapour interface and $\Delta\lambda$ is the 'thickness' of the interfacial region. We have taken $\Delta\lambda = 0.49$ nm (ref. 12). The relative pressure p/p_s was controlled by maintaining a constant temperature difference between the mica surfaces and a pool of liquid cyclohexane in the chamber of the apparatus. Errors in (p/p_s) are due to temperature drifts during an experiment. The principal errors in r_m arise from uncertainty in the thickness of the adsorbed film (about ±0.1 nm) and from errors in establishing the limits at which menisci were clearly observed to evaporate irreversibly (upper limit for D_c) or to grow (lower limit for D_c).

In conclusion, we have verified that the Kelvin equation for cyclohexane condensed on to mica surfaces is satisfied to within ±6% of r_m down to meniscus radii of 4 nm. A more detailed account of this work will be published elsewhere.

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Measurement of carbon tetrafluoride in the atmosphere

ATMOSPHERIC trace concentrations of carbon tetrafluoride (CF_4) in a variety of ambient air samples have been measured. The air samples were concentrated by a cryogenic trapping technique and the CF_4 species identified by combined gas chromatography–mass spectrometry (GC–MS) with single ion monitoring. The contribution of natural and/or anthropogenic sources to the global burden of CF_4 is unknown. CF_4 may be one of the atmosphere's rare gases.

CF_4 is a very stable compound that behaves like an inert gas up to a temperature of about 2,000 °C. The presence of CF_4 in the atmosphere at a concentration of approximately 0.1 to parts per 10^9 (p.p.b.) was deduced by Gassman[1] from MS analyses of contaminant levels of CF_4 in high-purity krypton samples. Gassman suggested[1] that CF_4 was enriched from the atmosphere during the cryogenic preparation of high-purity krypton. The GC–MS analyses made at Harwell were conducted to determine whether CF_4 is indeed a common constituent of the ambient atmosphere, and if so at what concentration.

A pre-concentration technique was used to provide samples for GC–MS analysis. A glass U-tube equipped with Teflon stopcocks was immersed in liquid nitrogen and air was allowed to liquify until the tube was approximately two-thirds full. Clean helium was then bubbled slowly through the trap until the liquid air had evaporated. The trap was then removed from the liquid nitrogen and thermally equilibrated at room temperature. This created a positive pressure of helium in the trap containing enriched concentrations of the trace species present in the air at the time of trapping. Samples of the mixture were then simply taken off the side arms of the trap with a syringe and injected directly into the chromatographic column. The helium gas used in the enrichment process was cleaned before passage into liquid air by passing through 40 ft of stainless steel tube ($\frac{1}{8}$ inch outer diameter), immersed in liquid nitrogen. This effectively removed all CF_4 and Xe that may have collected in the liquid air trap in the evaporation process. Analysis of blanks made by passing helium through the cold empty trap for 30 min showed no signs of either CF_4 or Xe.

For quantification both ^{84}Kr (boiling point −158 °C) and ^{132}Xe (boiling point −107 °C) were measured directly in 5 ml air samples and in the cryogenic preparations. Essentially 100%

enrichments of ^{84}Kr and ^{132}Xe were obtained. Accordingly the high efficiency of the cryotrap for ^{84}Kr and ^{132}Xe bracketing CF_4 with its intermediate boiling point of −128 °C was taken to indicate that CF_4 was quantitatively enriched. Subsequent analyses measured only ^{132}Xe for use as an internal standard in the CF_4 analyses. The concentration of ^{132}Xe in the air is 23 p.p.b. determined from its isotope abundance of ~27% of a total xenon atmospheric concentration of 84 p.p.b. Accordingly its constant atmospheric concentration provided an excellent internal standard for determining the enrichment ratios of the samples studied.

Analyses were performed on a Hewlett-Packard 5700 series gas chromatograph fitted with a $\frac{1}{4}$ inch o.d., 3 ft glass column packed with 70–140 mesh Woelm alumina GSC-120. Column temperature was held at −20 °C to enrich the 10 ml injections of the cryotrap contents onto the head of the column. After elution of the air peak, at 2 min, the temperature was raised ballistically (>32 °C min^{-1}) to +50 °C. Helium purified by passage through liquid N_2 was used as the carrier gas to 40 ml min^{-1}.

The gas chromatograph was interfaced to a V.G. Micromass-16 mass spectrometer by a jet separator. Mass 69 (CF_3) was monitored as the major peak for the parent CF_4 species with a retention time of 3.2 min. ^{132}Xe was measured at 4.2 min.

A representative analysis of CF_4 and ^{132}Xe chromatographed from an enriched air sample from South Pole, Antarctica, is shown in Fig. 1. The peak heights of each compound were used for quantitation against a CF_4 calibration standard in air and the known atmospheric concentration of ^{132}Xe. The ^{132}Xe peak was used as an internal standard to determine the enrichment ratio obtained in processing the cryotrap. The gains of the output attenuation of the strip chart record are CF_4, $10^{-8} \times 10$ and ^{132}Xe, $10^{-5} \times 5$ A V^{-1}.

The concentration of CF_4 was determined in 21 analyses of 11 different ambient air samples representative of the environs around AERE Harwell, and three other air samples representing the South Pole, New Zealand and the Pacific Northwest. The results of our measurements of the concentration of CF_4 are shown in Table 1. The mean CF_4 concentration for 16 analyses of Harwell air is 63.6 ± 13.2‰ by volume. The mean of the other three air samples for five analyses is 64.4 ± 3.2 p.p.b. by volume. No significant difference between the two sample populations is indicated. The variability in the Harwell values is about equal to the variability obtained in repetitive and/or duplicate analyses. Accordingly the CF_4 concentration in the Southern Hemisphere and that observed for the Northern Hemisphere suggest no inter-hemispheric differences at the present level of precision of analysis.

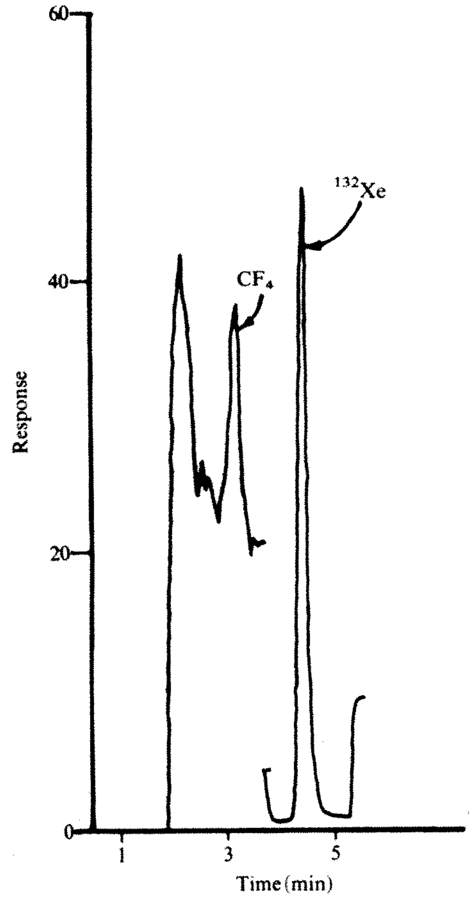

Fig. 1 GC–MS analysis of CF_4 and ^{132}Xe in air sample from South Pole, Antarctica.

Table 1 CF_4 Measurements

Date of analysis	Sample	Concentration factor	CF_4 (‰)*
		Harwell	
17/7/78	OA	30.8	56.5
17/7/78	OA	16.1	66
		20.3 repeat	42
17/7/78	OA	15.9	58
		16.3 repeat	55
17/7/78	OA	19.5	59
18/7/78	OA	30.95	55
		34.52 repeat	43
18/7/78	OA	25.97	83
18/7/78	OA	29.41	71
		116.18 repeat	85
18/7/78	OA	10.3	74
19/7/78	OA	36.62	83
19/7/78	OA	6.02	69
		5.26 repeat	56
19/7/78	OA	30.16	62
		Other	
19/7/78	PNW 171	8.20	63
		37.50 duplicate	67
20/7/78	S. Pole, Antarctica	15.04	64
		18.27 duplicate	60
	Godley Head, NZ	9.44	68
		Volcano	
19/7/78	Iilewa, Hawaii	11.27	
		6.27 duplicate	48
20/7/78	MLC, Hawaii	12.39	46
		6.81 duplicate	51

OA, outside air; MLC, Mauna Loa Cauldera; PNW, Pacific Northwest.

*Parts by volume.

At the same time as the CF_4 measurements of ambient air were made, two samples of volcanic emissions representative of a hot fumarole in the caldera of Mauna Loa (13,000 ft) and a much cooler vent on the north-east side of Kilauea at Iilewa (3,500 ft) were analysed. The two samples showed similar levels of CF_4 of 48.3 ± 2.5‰ by volume but at concentrations considerably lower than the level of 64‰ determined for the ambient air. The related halocarbon and other trace gas measurement data are given in Table 2.

The 25% lower level of CF_4 observed in the volcanic samples is consistent with the 17–27% lower fluorocarbon 11 and 12 levels typically observed in the Hawaiian volcanic emissions. This is interpreted to mean that the origin of both the fluorocarbon 11 and 12 and CF_4 is not from the gases being released from the fumarole's activity, but rather their presence represents entrainment of ambient air into the subterranean passages that is re-ejected into the atmosphere via the hot air plume in the chimneys associated with different volcanic vents. During the contact with the hot volcanic rocks some destruction of these materials has occurred as well as dilution. Previously Gassman had suggested that volcanic emissions may be a source of CF_4. This may still be correct as the Hawaiian volcanic emissions studied are not typical of the active magnetic gases released

Table 2 Trace gases in Hawaiian volcanic emis[illegible]ns

Sample	O_2* (%)	N_2* (%)	N_2O (p.p.b.)†	CO_2 (p.p.m.)†	F12 (‰)†	F11 (‰)†
Mauna Loa	100	100	840	3,960	211	123
Iilewa	100	100	380	360	220	140
Ambient air	100	100	330	328	275	168

*N_2 and O_2 percentages are derived from concentrations of nitrogen or oxygen in volcanic emissions over respective nitrogen or oxygen concentrations in ambient air.

†Parts by volume.

during a volcanic eruption. Also the halogen concentrations in the Mauna Loa type of volcanic emission are much lower than the halogen content of the emissions from other types of volcanoes.

The direct industrial production of CF_4 is minimal—<10 tons yr^{-1}. Therefore, its dispersive use is not expected to be a significant source of CF_4. The only other documented source of CF_4 is the electrolytic alumina reduction furnace. However, the production of CF_4 in this industrial process is not continuous, rather it is restricted to 5–10 min periods per day as the alumina content of the cell approaches depletion[2,3]. Because of its sporadic nature, any estimate of CF_4 emissions from the alumina reduction process is subject to a very large spread in the amount of CF_4 produced and released to the atmosphere. Nevertheless, however speculative, we estimate that about 6×10^9 g yr^{-1} of CF_4 may be emitted from the world wide 1977 production of aluminium of 1.34×10^{13} g yr^{-1}. If this is the case, then the aluminium industry is not likely to account for all of the global atmospheric burden of CF_4, which at an average concentration of 65‰, approximates to 10^{12} g. More information is obviously needed to establish the true contribution of CF_4 from the aluminium industry, but it is equally obvious that other sources of CF_4 need to be found.

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Trace metals in Antarctic snows since 1914

THE results of the variations of the concentrations of 12 elements including Pb, Cd, Cu, Zn and Ag in snow deposited from 1914 to 1974 at an uncontaminated site in East Antarctica are discussed here. As the composition of snow in central Antarctica is shown to be closely correlated to that in the lower atmosphere, this provides us with a valuable historical record of the composition of remote aerosols in the Southern Hemisphere. We find that Pb, Cd, Cu, Zn and Ag were already strongly enriched 60 yr ago (with respect to the composition of reference crustal and marine sources), and that both the concentrations and the enrichment factors observed in 1914 for these five elements are comparable to the present ones. This suggests that the present background atmospheric concentrations of Pb, Cd, Cu, Zn and Ag in the Southern Hemisphere are not strongly influenced by global pollution, but are related to natural phenomena, possibly volcanism.

A recent increase in studies of the chemical composition of tropospheric aerosols in remote areas has included data[1,2] from northern and southern regions, including the South Pole. Concentrations of heavy metals are found to be much greater than expected on the basis of the average composition of the Earth's crust and of seawater. We cannot determine whether this is due to man's increasing industrial activities by current atmospheric sampling programmes. However, we can determine the relative contributions to the composition of aerosols from natural and anthropogenic sources by analysis of impurities in successive layers of snow in polar ice sheets, as has been done for near surface layers of the Greenland ice sheet[3–6].

During the 1974–75 summer field season, 13 successive snow samples were collected from the surface to a depth of 5.35 m in a hand dug pit at Dome C (74°40′ S, 124°10′ E, elevation 3,240 m), which was at the time an uncontaminated site 600 km from the nearest scientific station and some 240 km from the nearest oversnow traverse. Samples were collected within a few weeks of the first aircraft landing at the site. The samples of the firn, about 0.7 kg each, were collected[7] by operators clothed in clean room garb, using Plexiglass tubes with screwed caps (inside diameter 8 cm, length 42 cm, rinsed in a class 100 clean room with ultrapure acids and water) pushed directly into the snow along a scoured pit wall. The tubes were then placed in double sealed polyethylene bags and kept frozen until the analytical stage. The samples were transferred by gravity directly into Teflon bulbs inside a class 100 clean room, for preconcentration[8] (×150) by non-boiling evaporation; this preconcentration procedure includes dissolving any solid particles by hot concentrated ultrapure hydrofluoric and nitric acids. Na, Mg, K, Ca, Fe, Al, Mn, Pb, Cd, Cu, Zn and Ag are then determined by graphite furnace atomic absorption (electrodless discharge lamps for Pb, Cd and Zn) in the clean room. The complete procedure was calibrated[8] by processing 80 synthetic standard solutions of various concentrations down to 10^{-12} g/g that overlapped the Antarctic snows concentration ranges. Contamination from the field Plexiglass sampling tubes was found to be negligible for all the 12 elements[7] by analysing ultrapure water used for an extensive rinsing of 'blank' field Plexiglass tubes. The overall analytical precision (atomic absorption and preconcentration process) calculated from the calibration curves using

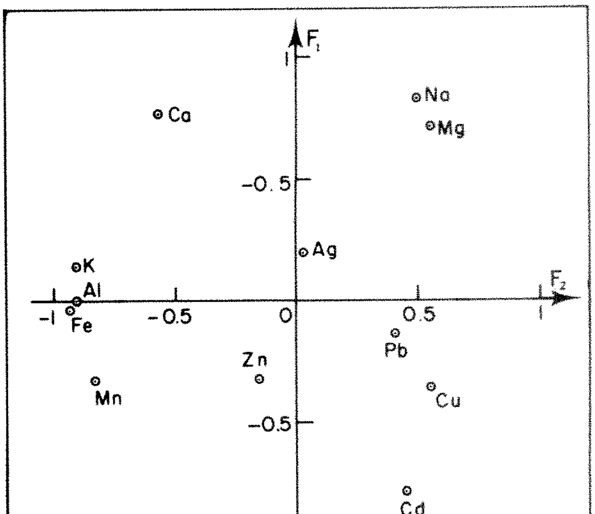

Fig. 1 Dome C, Antarctica: factor analysis. Two dimensional plot using eigen vectors F_1 and F_2.

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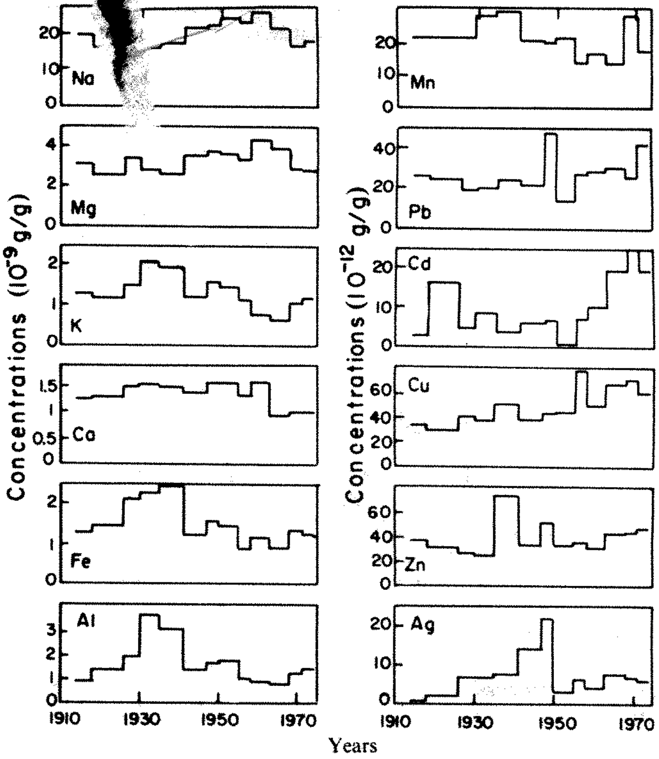

Fig. 2 Dome C, Antarctica: variations of the measured concentrations in snow between 1914 and 1974.

confidence limits of 95% is of the order of 10% (30% for Cd). All data are summarised in Table 1.

The age of the snow samples collected between the surface and 2.06 m has been determined in our laboratory by various independent techniques[9] including stratigraphy, stable isotope composition and radioactive fallout horizons from nuclear tests, which identify seasonal snow layers and make it possible to attribute specific dates to several characteristic horizons over the past 25 yr. Beyond 2.06 m, solely stratigraphic data have been used. The age of the samples ranges from 1974 to 1914, Table 1. The accuracy of these unpublished dating results (Petit, personal communication) is ±1 yr between the surface and 2.06 m (years 1974–55); it decreases then with increasing depth to ±5 yr at 5.35 m (year 1914).

The elements measured in this work can originate from three principal sources: the oceans, the continents and man's activities. The relative influence of these three sources at Dome C can be determined by analysing the correlation matrix of the 12 measured elements through principal components factor analysis[10,11] (FA). FA studies this correlation matrix by diagonalising it, solving it for eigen values and vectors which are linear combinations of the original 12 elements, and taking these eigen vectors as new reference axes. The results of FA are shown in Fig. 1 for the two eigen vectors $\mathbf{F}_1$ and $\mathbf{F}_2$ corresponding to the two highest eigen values; these two vectors account respectively for 40.6 and 23.5% of the sample set variance. They separate three groups of elements: Na (marine reference element) and Mg (top right); Al (crustal reference), Fe, Mn, K and Ca (left); Pb, Cd, Cu, Zn and Ag (bottom right). This suggests an oceanic source for Na and Mg, a crustal continental source for Al, Fe, Mn, K and Ca, and a third source, independent from the two others, for Pb, Cd, Cu, Zn and Ag, as previously found at South Pole Station[12].

It has been suggested[13] that in clean air conditions, the chemical composition of precipitations and of aerosols would be closely related. This is seen to be the case at the South Pole as shown by the data of Table 2. The two sets of data do not correspond to comparable time periods: this is probably unimportant as only weak seasonal variations of the concentrations are observed[10] in these areas, at least in snow, but for Na and Mg. The ratio of the concentrations measured in air (expressed in 10^{-12} g per standard cubic metre) to those measured in deposited snow (expressed in 10^{-12} g/g) range from 0.4 to 1 but for K and Cd for which slightly higher ratios are obtained. Although this relationship might be influenced by various parameters, it is unlikely that marked changes happened over the past century which was characterised by rather constant climatic conditions. The variations of the trace elements content of dated snow layers in central Antarctica can therefore probably be used to reconstruct the past variations of the aerosol content of the low troposphere. Variations in concentration of all 12 elements over the period 1914–74 are shown in Fig. 2.

At Dome C there are significant variations with time for all the elements. These variations are probably not linked with short-term changes such as seasonal effects[4,10], as each sample integrates at least 4 yr of snow accumulation. Generally speaking, concentrations in firn of 60 yr age are similar for all the elements to the present concentrations, except perhaps for Cu, whose concentrations increase slightly (×1.5) between 1914 and 1974. Apparently, therefore, the atmospheric concentrations of the measured elements have not increased markedly, despite significant variations, since 1914. We previously found[12] an increase of the concentrations of Pb, K, Zn and Ag (×2–3) and Cu (×6) in snow between 1950 and 1974 at South Pole Station. The present data obtained at Dome C confirm this increase between 1950 and 1974 for Pb, but the increase is less pronounced for Zn, Ag and especially Cu at Dome C than at South Pole Station; no increase is detected for K. This suggests that slight local contamination associated with operations at South Pole Station, which has been occupied since 1957, may have occurred for some elements.

The absence of an increase in the atmospheric concentrations of trace elements at Dome C between 1914 and 1974 suggests that they are not significantly affected by global pollution.

Table 1 Dome C, Antarctica: measured concentrations in snow

Depth	Years	Na	Mg	K	Ca	Fe	Al	Mn	Pb	Cd	Cu	Zn	Ag
(m)				(10^{-9} g/g)						(10^{-12} g/g)			
0.10–0.48	1974–71	17.9	2.77	1.06	1.00	1.24	1.14	18	41	19	62	47	6.0
0.48–0.86	1970–68	17.0	2.82	0.93	0.99	1.36	1.25	29	25	25	72	45	6.8
0.86–1.25	1967–63	21.7	3.93	0.62	0.94	0.91	0.78	14	30	19	69	43	7.6
1.25–1.64	1962–58	26.1	4.32	0.75	1.51	1.16	0.88	17	28	8.9	50	31	4.0
1.64–2.06	1957–55	23.5	3.31	1.11	1.32	0.88	0.97	14	27	6.8	80	36	6.6
2.06–2.50	1954–50	24.8	3.61	1.40	1.55	1.46	1.81	22	15	1	45	34	2.9
2.50–2.90	1949–47	23.7	3.71	1.57	1.53	1.57	1.70	20	47	6.7	44	54	22
2.90–3.33	1946–41	21.7	3.52	1.13	1.36	1.22	1.37	21	21	6.1	39	35	14
3.33–3.75	1940–35	17.4	2.57	1.88	1.47	2.42	3.06	30	23	3.3	52	74	7.2
3.75–4.15	1934–30	16.1	2.78	2.05	1.52	2.25	3.66	29	20	8.3	38	26	6.5
4.15–4.55	1929–26	21.2	3.41	1.45	1.47	2.11	1.93	22	18	4.8	41	27	6.6
4.55–4.95	1925–18	16.2	2.48	1.12	1.27	1.47	1.39	22	24	16	29	32	2.1
4.95–5.35	1917–14	19.8	3.11	1.25	1.24	1.28	0.95	22	26	2.27	34	38	0.2

Historical records of some trace metals discussed here have already been published for Greenland in the Northern Hemisphere[3–6]. No trend was found for Hg [5] and Cd [4,6], while Pb [3,4] and Zn [4] concentrations were shown to have increased by a factor of two to three since the beginning of the century. There was no clear results for Cu [6]. Our results therefore indicate that industrial pollution has had no significant effect on the background composition of the atmosphere of the Southern Hemisphere, although it seems to have some effect on the background content of some heavy metals (Pb, Zn) in the Northern Hemisphere atmosphere. This is in good agreement with the fact that more than 90% of anthropogenic aerosols are emitted in the Northern Hemisphere[15], and that the Equator is a barrier for tropospheric aerosols.

The interesting features, shown in Fig. 2, are the high measured concentrations of Al, Fe, Mn and K (elements of crustal continental origin according to FA) between 1925 and 1940 approximately. This could be explained by an enhancement of the meridional circulation between 1925 and 1940 in the Southern Hemisphere, resulting in an increase of the transport fluxes of crustal material from mid latitude continents to Antarctica. We have no literature values on the historical variations of the circulation patterns in the Southern Hemisphere, but it has been suggested[16] that, in the Northern Hemisphere, they were different between this time period and the remaining other years of the 1900–68 period.

The variation with time of the enrichment factors with respect to bulk seawater (EF_{sea}) for Mg, and with respect to the mean crustal composition[17] (EF_{crust}) for K, Ca, Fe, Mn, Pb, Cd, Cu, Zn and Ag are shown in Fig. 3. The reference elements are respectively Na and Al. The precision is better than 20% for all the elements except for Cd (40%). Calculated EF_{sea} values are close to unity for Mg, confirming that the sea is the likely source for this element. EF_{crust} values near unity are obtained for K, Ca, Fe and Mn suggesting again a crustal continental source for all these elements.

On the other hand, high EF_{crust} values ranging from one to four orders of magnitude are observed for Pb, Cd, Cu, Zn and Ag. This supports the FA data showing an independent source for these last elements. For Pb, Cd, Cu and Zn, the EF_{crust} decrease more or less sharply from 1914 to 1930; they remain then almost constant between 1930 and 1950, and then increase from 1950 to 1974. There are large variations for Ag, but the mean values between 1914 and 1930 are not very different from those between 1950 and 1974. It seems that despite large variations with time, the enrichments factors for the heavy metals are as high in 1914 as the ones observed in 1974. This again suggests that these high enrichments observed for heavy metals in Antarctic snows, and therefore for the Southern Hemisphere background aerosols, are not significantly influenced by global pollution, but are linked with natural phenomena.

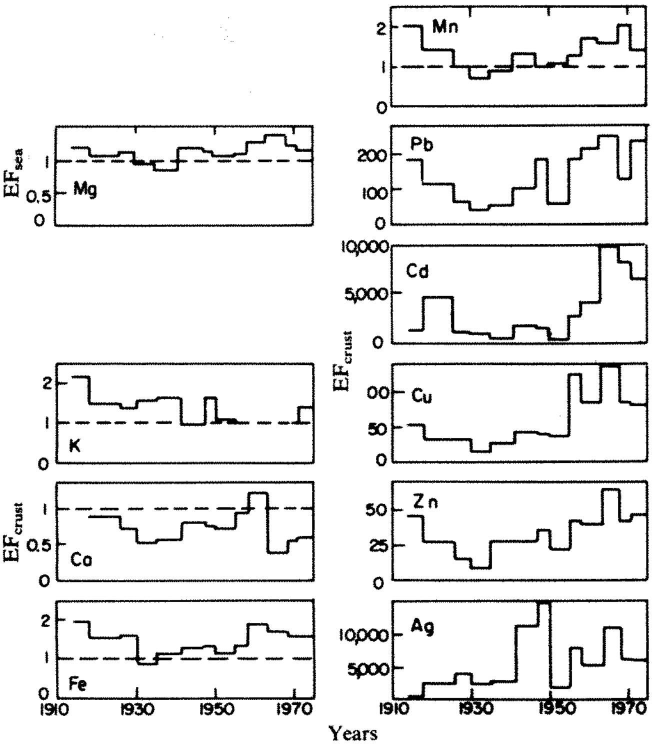

Fig. 3 Dome C, Antarctica: variations of the enrichment factors between 1914 and 1974.

Table 2 South Pole station: comparison of the chemical composition of deposited snow with that of local atmospheric aerosols

Element	Concentrations Aerosols* (10^{-12} g/standard cubic metre)	Snow† (10^{-12} g/g)
Na	3,300 ± 1000	7,700 ± 140
Mg	720 ± 430	1,300 ± 130
K	680 ± 140	370 ± 70
Ca	490 ± 200	510 ± 190
Fe	620 ± 230	760 ± 90
Al	820 ± 380	1,070 ± 250
Mn	13.5 ± 3.5	18 ± 4.5
Pb‡	27 ± 10 76 ± 40	40 ± 11
Cd	18 ± 13	3.8 ± 2.8
Cu	29 ± 17	47 ± 1.4
Zn	33 ± 20	6.8 ± 7.8
Ag	3.4 ± 3.6	5.5 ± 0.07

* From ref. 14. The results are the average of five sampling periods of nine days between 16 December 1974 and 4 February 1975. The error listed is the standard deviation derived from the dispersion of the concentrations for the five sampling periods.

† From ref. 10. The results are the mean concentrations measured in four snow samples integrating the years 1973 and 1974. The error listed is the standard deviation derived from the dispersion of the concentrations for the four samples.

‡ The two values for aerosols correspond to two sets of samples collected by using different sorts of filters (see ref. 14).

The possible natural sources or mechanisms responsible for these very important enrichments of heavy metals are still being discussed[1,2,4,18]. The emission into the atmosphere of heavy metals from the ocean surface microlayer[19,20] and from plants[21,22] has been described and could be very significant. However, the most likely source could be volcanism. Little information is available on the chemical composition of volcanic aerosols, but it seems that they are strongly enriched in Pb, Cd, Cu, Zn and Ag[23,24], with enrichment factors close to those determined at Dome C[23,24]. The background fallout fluxes of these five elements for the Southern Hemisphere calculated[25] from our Dome C data (by assuming as a first crude approximation an homogeneous deposition pattern) correspond approximately to emission fluxes[24] from about 10 volcanoes similar to Mt Etna in Italy. Moreover, the historical record of the stratospheric burden of volcanic aerosols[26] shows that after decreasing from 1900 to 1920, and being low between 1920 to 1945, this burden increased after 1945. These time variations are similar to observed changes of concentrations shown in Fig. 2 and enrichment factors shown in Fig. 3 for Pb, Cd, Cu, Zn, but not for Ag. Although it has been shown[27] that remote volcanic aerosols can reach the Antarctic ice cap, more detailed information on volcanic sources (chemical composition, fluxes) and analyses of snow samples covering a much longer time period including more particularly the years 1850–1910 which were characterised by the two powerful eruptions of Krakatoa (1883) and Santamaria (1903) are needed to confirm if volcanoes are really responsible for the past and present day atmospheric concentrations of heavy metals.

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Gerontology of the Allende meteorite

IN the Allende meteorite several elements are found to have an isotopic composition that cannot be due to radioactive or spallation or fractionation processes[1]. These isotope anomalies are mostly confined to white inclusions enriched in refractory elements[2] (Ca–Al-rich inclusions) and are thought to be introduced into the Solar System by precondensed grains[3]. We have previously reported an anomalously high K–Ar age of a composite sample of five white inclusions from the Allende meteorite[4]; no direct verification of this lone-standing result of 4,800 Myr was possible because, with the exception of a small chip of one of the inclusions used for a thin section, the material was fused in the ^{40}Ar–^{39}Ar experiment. We therefore began a search for old material in other Ca–Al-rich inclusions. Three different samples from a new type of inclusion proved to be no older than most of Allende's constituents, 4,510 Myr (ref. 5). A small white inclusion gave a well developed ^{40}Ar/^{39}Ar plateau over almost the entire ^{39}Ar release, with an age of 4,500 ± 20 Myr (ref. 6). We report here on the ^{40}Ar–^{39}Ar analysis of two further coarse-grained Allende inclusions that showed ages in excess of 4,550 Myr.

In the most widely accepted theory a supernova triggered the collapse of the solar nebula[3,7] and the anomalously high ages would be due to an enhanced ^{40}K/^{39}K isotopic ratio produced in the explosive carbon burning shell of the supernova[7]. In another, controversial interpretation these ages could have chronological significance, as here the presolar grains are relicts from various old stellar nucleosynthetic and condensation processes unrelated to the formation of the Solar System[8-10].

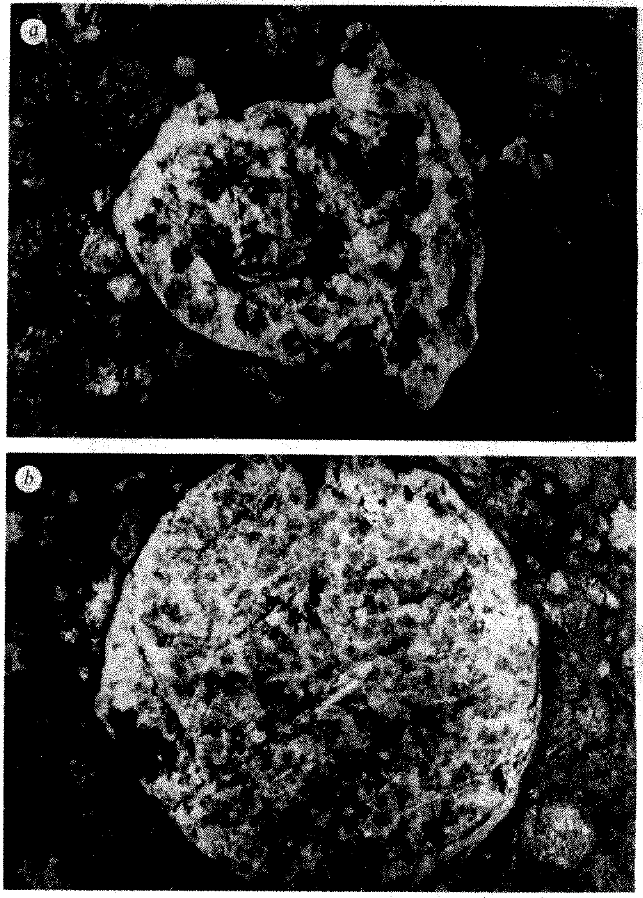

Fig. 1 Photograph of two Allende inclusions from which chips were taken for ^{40}Ar–^{39}Ar analysis. (Sample 17(*a*); sample 18(*b*)).

Sample 17 was a 15.1 mg interior chip of an irregular white cavernous inclusion (Fig. 1*a*). Binocular inspection revealed the presence of at least three different mineral phases: dark grey and clear crystals (up to 1 mm) were closely packed in a sugary white matrix material. The inclusion was surrounded by an 80–100 μm wide, extremely fine-grained rim locally enriched in spinels.

Sample 18 was a 40.9 mg interior chip of a round white inclusion (Fig. 1*b*) with a diameter of ~11 mm, which also had a core and a rim (0.2 mm). The core consisted of dark crystals (0.5 mm) embedded in a light grey crystalline matrix. Microscopic investigation showed a mineral composition typical of type B inclusions[2]. The core of the inclusion consists of prismatic xeno- and hypidiomorphic clinopyroxene crystals (80 μm–1 mm, majority 200–500 μm). Melilite and anorthite are subordinately present, melilite as xeno- or hypidiomorphic, sometimes elongated grains (350 μm–2 mm), anorthite as lath-shaped twinned crystals. Spinel occurs either as hypidio- and idiomorphic isolated grains (2–25 μm), poikilitically enclosed in pyroxene, melilite, and anorthite, or as round aggregates (30–160 μm) of minute crystals. Inclusions of opaque minerals dispersed in pyroxene and melilite are common. The rim of the inclusion, 80–100 μm wide, consists of extremely fine-grained crystalline material, partly enriched in spinels (5–15 μm). Some pyroxene crystals show deformation lamellae. More detailed mineralogical as well as chemical studies of these inclusions are in preparation.

The irradiation, argon extraction, purification, and data

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analysis procedures are identical to those previously published[11], except that the atmospheric argon was not removed before the ^{40}Ar–^{39}Ar experiment by preheating.

The results of the argon analysis are given in Table 1, at the same stage of data reduction as in ref. 11. For the apparent ages (I), a correction for trapped ^{40}Ar with the conventional ratio $(^{40}\text{Ar}/^{36}\text{Ar})_{\text{trapped}} = 1 \pm 1$ was applied. To take into account atmospheric contamination, minimum ages (II) were calculated after subtraction of $295.5 \times {}^{36}\text{Ar}_{\text{trapped}}$ (that is, in the atmospheric ratio) from ^{40}Ar. These ages are the basis of our present discussion. Age spectra are shown in Fig. 2.

Absolute concentrations are 155 p.p.m. K, 12% Ca and 70 p.p.m. K, 14% Ca for sample 17 and 18, respectively. In the reactor irradiation ^{38}Ar is produced from Cl present in small amounts[12] and thus nominal ^{38}Ar/Ca exposure ages in these inclusions are too old compared with the known cosmic ray exposure time of Allende of 5 Myr (ref. 13). Note, however, that ^{38}Ar from Cl is small in the 1,130–1,380 °C fractions of sample 17, which yields a weighted average exposure age of 3.9 Myr (production rate 2.8×10^{-3} ccSTP $^{38}\text{Ar}_{\text{C}}$ per g_{Ca} Myr).

The age spectra obtained from both samples are rather similar. Taking the minimum apparent ages, we observe broad plateaus extending over the first 50–60% of ^{39}Ar released. At high extraction temperatures, beginning at 1,070 and 1,300 °C, respectively, the apparent ages become older by about 400 Myr, compared with the plateaus.

The two-step patterns of the age spectra are reflected in the K/Ca variations. K/Ca of the plateau fractions is relatively stable, most pronouncedly so in sample 17, indicating that over a wide temperature regime the same mixture of phases is degassed. Low K/Ca ratios at higher temperatures are associated with high ages, the opposite of what is expected from ^{39}Ar recoil[14].

Two-step ages patterns have also been previously encountered in lunar highland breccias[11,15,16]. There the significance of both parts of the spectra had been confirmed by spotwise laser ^{40}Ar–^{39}Ar dating[17,18]. The difference between the results of those lunar studies and of Allende samples 17 and 18 is the >4,500 Myr age of the latter and the 'normal' ages of the former. In addition to the samples reported here, we irradiated and analysed a sample of lunar highland breccia 73215, 170, 6, a pink spinel-bearing troctolitic clast (O. B. James, personal communication), which below 1,050 °C yields a plateau age of 3,940 ± 70 Myr in agreement with the formation age of the breccia of 3,870 Myr (ref. 19). At 1,400 °C half of the ^{39}Ar is released with an apparent age of 4,450 ± 40 Myr. This age is about the same as obtained for other early lunar differentiates[15,20]; it may even be the oldest one. The age of this clast fits perfectly into the chronological framework established for breccia 73215 (refs 11, 19, 21). It also demonstrates that the >4,500 Myr ages of our two Allende inclusions are not due to some kind of a calibration error. Instead, the new results support the 4,800 Myr K–Ar age previously reported. The nominal K–Ar ages of samples 17 and 18 are 5,120 ± 60 Myr and 5,080 ± 50 Myr, and the plateau ages are 4,980 ± 60 Myr and 4,900 ± 30 Myr, respectively. A high-temperature phase must be responsible for the still higher apparent ages of the 1,130 or 1,300 °C fractions, averaging 5,400 ± 100 Myr.

The ratio of 'excess' ^{40}Ar (over that produced since 4,550 Myr) to K is 0.033 ccSTP per g for both samples. It is unlikely that this excess ^{40}Ar migrated from the K-poor matrix (200 p.p.m. Jessberger *et al.*, in preparation) into the inclusions. Not only is there no precedent for such a migration, but it would also have to occur to precisely the right degree to balance the different K concentrations.

It has long been argued that the isotopic anomalies and some other peculiar features of the Ca–Al-rich inclusions were introduced into the Solar System by presolar grains[8,22–25]. Two principal origins of the presolar grains have been proposed. According to Clayton[9], they represent interstellar dust from various sources. Such dust, if not outgassed, should have a mean K–Ar greater than that of the Solar System. In that picture[10], the anomalously old ages are real and indicate the survival of

Table 1 Amounts and isotopic composition of Ar released from two coarse-grained white inclusions of Allende after experimental corrections. Apparent ages II are calculated after subtraction of $295.5 \times {}^{36}\text{Ar}_{\text{T}}$ from ^{40}Ar to account for atmospheric contamination

Extraction temperature (°C)	^{40}Ar (10^{-8} ccSTP per g)	^{36}Ar/^{40}Ar	^{37}Ar/^{40}Ar	^{38}Ar/^{40}Ar	^{39}Ar/^{40}Ar	Apparent age (10^3 Myr)	
		(×10^5)	(×10^3)	(×10^5)	(×10^5)	I	II
a Allende no. 17 (15.1 mg)							
500	117	301 ± 6	32 ± 1	102 ± 5	99 ± 4	7.68 ± .08	4.96 ± .42
580	79	215 ± 7	55 ± 1	93 ± 5	283 ± 13	5.83 ± .08	4.60 ± .16
660	140	139 ± 4	54 ± 1	121 ± 4	374 ± 9	5.35 ± .04	4.97 ± .06
740	114	107 ± 2	77 ± 3	133 ± 4	436 ± 6	5.09 ± .02	4.97 ± .04
820	99	111 ± 3	156 ± 2	146 ± 5	439 ± 11	5.08 ± .04	4.99 ± .06
890	116	146 ± 2	162 ± 2	227 ± 5	408 ± 10	5.21 ± .04	5.21 ± .04
950	99	158 ± 7	133 ± 1	383 ± 8	470 ± 16	4.97 ± .06	4.97 ± .06
1,010	165	231 ± 5	116 ± 1	578 ± 7	470 ± 7	4.97 ± .03	4.97 ± .03
1,070	93	286 ± 3	286 ± 3	535 ± 14	377 ± 8	5.34 ± .04	5.34 ± .04
1,130	945	64 ± 4	1,104 ± 1	69 ± 4	335 ± 4	5.54 ± .02	5.43 ± .04
1,200	140	278 ± 15	3,327 ± 13	232 ± 13	227 ± 13	6.21 ± .10	5.25 ± .24
1,280	31	498 ± 36	9,540 ± 40	221 ± 34	411 ± 46	5.19 ± .19	1.46 ± 1.48
1,380	52	667 ± 47	14,050 ± 70	598 ± 52	130 ± 40	7.20 ± .59	2.67 ± 5.65
Total	2,189	156 ± 2	1,209 ± 2	188 ± 3	344 ± 4	5.50 ± .02	5.12 ± .06
b Allende no. 18 (40.9 mg)							
450	298	339 ± 1	—	75 ± 2	15 ± 1	11.01 ± .06	4.52 ± 2.24
580	116	313 ± 3	9 ± 1	82 ± 3	80 ± 6	8.06 ± .14	4.47 ± .50
670	145	290 ± 3	15 ± 1	96 ± 2	109 ± 2	7.50 ± .04	4.97 ± .16
750	124	243 ± 4	41 ± 1	117 ± 2	215 ± 3	6.31 ± .03	4.90 ± .09
830	78	193 ± 3	76 ± 1	237 ± 6	370 ± 4	5.37 ± .02	5.13 ± .06
900	45	224 ± 9	112 ± 1	629 ± 3	471 ± 11	4.96 ± .04	4.96 ± .04
970	39	312 ± 7	160 ± 2	1,027 ± 10	490 ± 13	4.90 ± .04	4.90 ± .04
1,040	45	372 ± 9	225 ± 2	1,359 ± 11	492 ± 11	4.89 ± .04	4.89 ± .04
1,110	37	404 ± 5	422 ± 2	1,487 ± 9	489 ± 9	4.90 ± .03	4.90 ± .03
1,190	101	355 ± 6	1,019 ± 3	990 ± 6	514 ± 5	4.82 ± .02	4.82 ± .02
1,300	318	710 ± 22	6,968 ± 7	1,760 ± 20	372 ± 21	5.37 ± .10	5.37 ± .10
Total	1,345	401 ± 5	1,761 ± 2	685 ± 5	250 ± 5	6.05 ± .04	5.08 ± .05

non-degassed K-bearing grains 'from the beginning of the Universe' through the turbulences of the Ca–Al-rich inclusion-forming processes. Irradiation of the presolar grains with cosmic ray particles before formation of the Solar System is proposed by Clayton's model. The absence of large amounts of spallation ^{38}Ar, however, does not necessarily invalidate this model, as the size of interstellar grains[26] is not sufficient to stop (and hence trap) the recoiling spallation products within the boundaries of the grains.

According to the second model, which is not in all aspects contradictory to the first, the presolar grains condensed in a late supernova that triggered the collapse of the solar nebula[3,7]. In this model apparent K–Ar ages can result from an isotopic enhancement of ^{40}K. Note here that K–Ar ages are usually not based on ^{40}Ar/^{40}K ratios, as the principle of the method would require, but in the case of the ^{40}Ar–^{39}Ar method they are calculated from ^{40}Ar/^{39}K ratios, which are converted to the proper ratios by assuming that the sample has the normal Solar System ratio of ^{40}K/^{39}K. As calculated by Cameron and Truran[7], the explosive carbon burning phase of supernovae produces a ^{40}K/^{39}K ratio that is 70 times the average Solar System ratio 4,600 Myr ago.

Other anomalies like the ^{48}Ca enhancement[27] are also attributed to the explosive carbon burning phase as are FUN anomalies[28,29]. An apparent K–Ar age of 5,100 Myr corresponds to a ^{40}K enrichment of 35%, whereas an age of 5,400 Myr as measured in the high temperature fractions of samples 17 and 18, requires a 60% enrichment of ^{40}K relative to the Solar System value of ^{40}K/^{39}K. These large enrichment factors, however, do not imply extraordinarily large numbers of extra ^{40}K atoms. The magnitude of this anomaly is only 1/50 of the excess ^{48}Ca atoms in inclusion EK 1-4-1 (ref. 27).

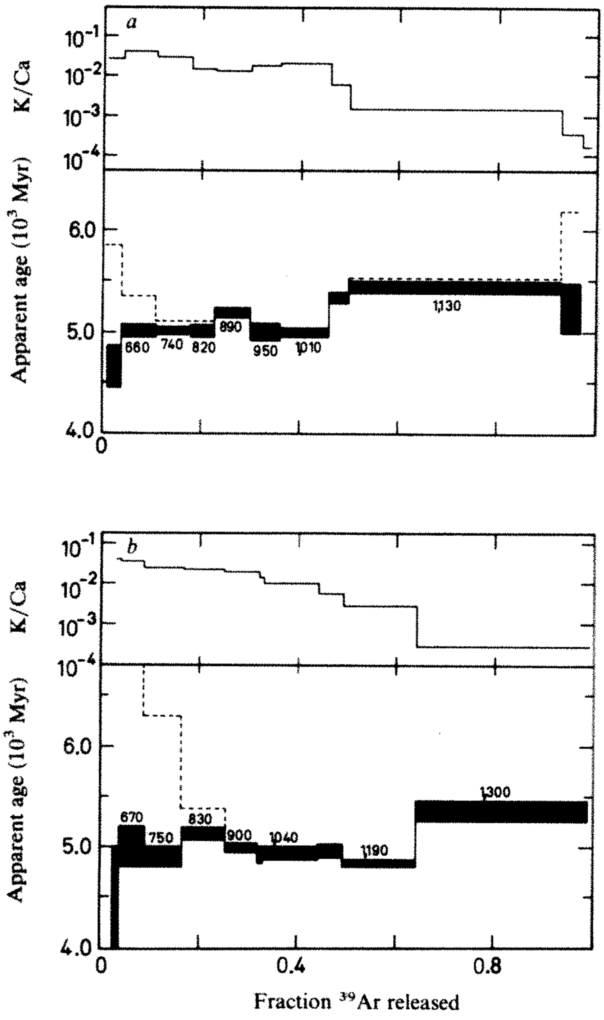

Fig. 2 K/Ca and apparent age spectra of two white Allende inclusions. The broken lines in the age spectra represent the apparent ages without correction for atmospheric contamination.

Unfortunately, the possible presence of a ^{40}K isotopic anomaly does not clearly distinguish between the two models, as both allow for ^{40}K enhancements. However, Clayton[10] predicts an additional ^{41}K anomaly in his model. This letter does not attempt to discuss the merits or problems of both models.

Up to now three Ca–Al-rich inclusions have been analysed for their K isotopic composition and have been found to have normal ratios[30,31]. These negative results, however, do not preclude samples 17 and 18 from having anomalous K. Note that so far, FUN anomalies of Mg, O (refs 28, 32) have only been encountered in two inclusions of Allende out of some 50 searched. Thin section studies of the parent inclusion of sample 18 revealed the peculiar feature of magnetite preceding the iron-phase condensation (A. El Goresy, personal communication). Samples 17 and 18 may represent new examples of presolar FUN material.

The experimental verification of gas retention ages older than the accepted age of the Solar System in itself should not be interpreted as evidence for the survival of presolar grains—although the 'ages' are highly suggestive—until the carrier material of the extra ^{40}Ar is identified and further isotopic evidence obtained.

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Continental geotherms during the Archaean

PRESSURE and temperature data from Archaean terrains have been used to test whether heat production during the Archaean at about three times the present rate resulted in correspondingly steeper continental geothermal gradients[1–6]. The rocks considered here lie mostly in the range of medium pressure metamorphism, recording pressures of 7–13 kbar at temperatures of 600–900 °C (ref. 2). These pressures, with the underlying, normal, thickness of crust imply great thickening of continental crust during the Archaean.

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Although these temperatures and pressures may record conditions during a phase of crustal generation—presumably dominated by igneous activity—the time for thermal equilibration of such a province is short (~50 Myr: for example, oceanic lithosphere) and unless the ages of the mineral parageneses can be tied in with this accuracy to phases of crustal generation, the crustal thickening and *P–T* data may result from a later and quite separate metamorphic episode.

By analogy with the behaviour of Phanerozoic terrains, most of the exhumation of these rocks must have occurred in the first few 100 Myr after the episode of crustal thickening. In these circumstances a *P–T* estimate represents only a point in *P–T*-time space and it is of little use, therefore, to try to fit it to any steady-state geotherm[7,8], particularly to one for normal crustal thickness.

However, it will be shown that if the time-dependent nature of the geotherm is accounted for, these data are still useful in that they give an upper limit to the geothermal gradient in thickened Archaean crust[5,6]. This is self-evident if the overthick crust was produced by magmatic intrusion, but it needs to be demonstrated if the thickening was not accompanied by extensive magmatism.

In the first case, the intrusive terrain would have cooled rapidly so that the *P–T* data reflect closely the magma-steepened geotherm, even when erosion is rapid[7]. If, however, the overthick crust was produced by over- or under-thrusting this will not necessarily be true. The common feature of these forms of crustal thickening is the lateral injection of cool crustal material, rather than the vertical emplacement of hot fluid. This results in the establishment of geotherms with dT/dP less than equilibrium ones, and their persistence for several tens of Myr (ref. 9); similar conditions apply if the overthick crust is produced by rapid sedimentation.

Erosion can considerably steepen the geotherm, however, and this, with continuing movement towards equilibrium during erosion, may mean that rocks buried deeper than about 30 km will not begin to cool until they have been decompressed by several kbar (refs 7, 10).

This point is demonstrated in Fig. 1 for two hypothetical terrains in which there is a linear gradient with the rock considered (initially buried at 40 or 60 km) at 900 °C. These profiles are then eroded at a linear rate[11] so that the rocks reach the surface in 50 or 200 Myr. The *P–T* paths for these rocks are shown in Fig. 1; when the rock is exhumed from 60 km between 80 and 90% of its cooling occurs in the last 30 km of its ascent and when it is exhumed from 40 km, the last 20 km of ascent shows 70–85% of the cooling. The portions of the *P–T* paths characterised by nearly isothermal decompression would be extended to lower pressures if there were radiogenic elements in the pile, as heating would continue during the ascent (see refs 7, 10). Also plotted in Fig. 1 are *P–T* data for the metamorphism of Archaean granulites summarised by Tarney and Windley[2]; a mean crustal density of 2.85 Mg m^{-3} is assumed in converting pressures to depth.

If the initial geotherms (dotted lines in Fig. 1) represent thermal profiles at, say, 70% of the temperatures which would be supported at equilibrium, then the dashed lines (E) show this equilibrium profile; probable *P–T* data from these rocks would lie close to (40 km burial) or to the high temperature side (60 km burial) of these equilibrium profiles. These arguments suggest that, whether the Archaean *P–T* data result from thickening of the crust by magmatism or from compression of cooler crustal material, they almost certainly represent temperatures as high as, or higher than, those which could be maintained at equilibrium in the thickened Archaean crust.

To estimate a 'normal' Archaean continental geotherm from the *P–T* data, thermal profiles for the thickened crust are calculated. It is assumed that a quantity of radiogenic heat production q_A, is distributed—either uniformly, or exponentially decreasing with depth on a scale length of 10 km—through the burden overlying the rocks which now yield the *P–T* data. The temperatures are sustained by this heating and a deep-seated contribution, q_*, from the lower portions of the crust and the mantle. The conductivity, *K*, is assumed constant throughout. A temperature of 900 °C at 40 km is taken as representative of the granulite *P–T* data; from the arguments given above this temperature is likely to be no lower than the equilibrium temperature at that depth in the overthick crust.

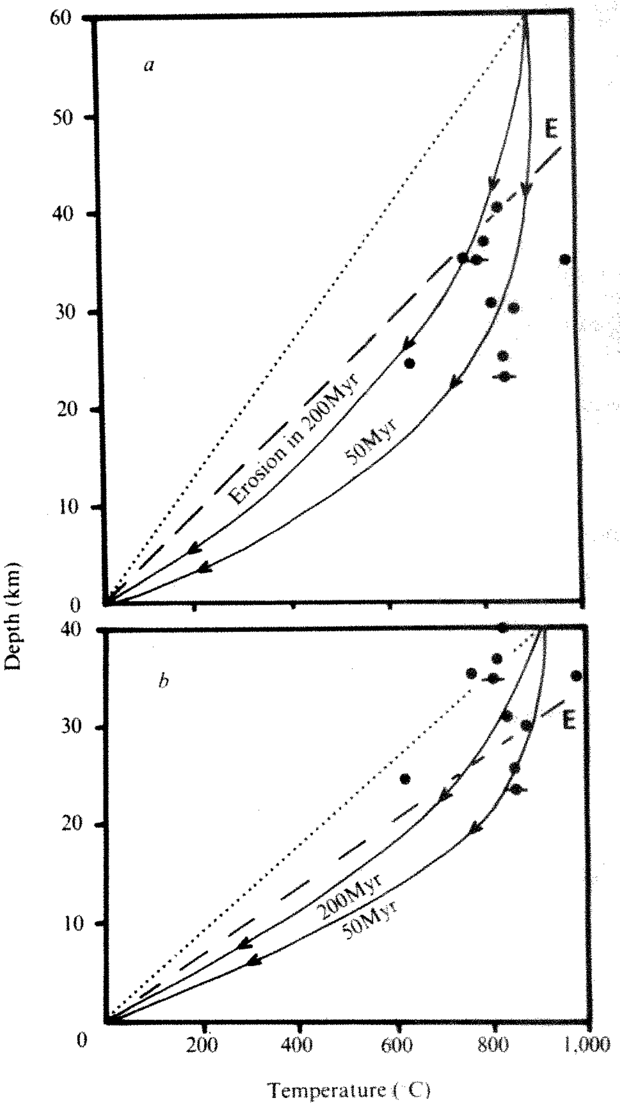

Fig. 1 *P–T* paths for rocks buried at depths of 60 km (*a*) and 40 km (*b*) in terrains of initially uniform thermal gradient such that they are at 900 °C at these depths. The rocks are subsequently exposed by erosion at a uniform rate over 50 or 200 Myr. Temperatures are calculated from Carslaw and Jaeger[11]. ● Indicate *P–T* estimates from Archaean terrains[2]; pressure and temperature ranges are plotted at their mid-points; and ● indicate that the pressure estimates are minima. Dotted lines (E) are 'equilibrium' geotherms if initial gradients represent only 70% of equilibrium temperatures.

An upper limit on the mantle contribution to Archaean continental geothermal gradients may be obtained by assuming that these temperatures were maintained without the help of radiogenic heating in the crust. The average crustal thermal conductivity is unlikely to have been greater than 4 W m^{-1} K^{-1} and this yields a limit of 90 mW m^{-2} for the Archaean mantle contribution to surface heat flow. In these conditions the temperature at the base of 35-km thick crust would be around 800 °C.

The heat production distribution in the overburden to the rocks preserving Archaean *P–T* data is, naturally, indeterminable and no more rigorous limit may be placed on q_* from these data. However, a plausible lowest limit on the radiogenic heat production in Archaean continental rocks may be obtained from measurements on the Th- and U-depleted pyroxene granulites from Australian Archaean terrains[12] and from measurements on high grade rocks in the Canadian Shield (see ref. 13). These rocks, after allowance for the decay of heat producing elements since the Archaean, would generate at least 1 μW m^{-3}, or a total of 40 mW m^{-2} through 40 km of overburden.

Table 1 shows the value of q_* required to maintain 900 °C at 40 or 60 km for a given value of q_A-distributed exponentially or uniformly through the 40 or 60 km of cover. Crustal radiogenic heat production at 40 mW m^{-2} over 40 km of cover requires q_* of 70–80 mW m^{-2}; some of this must come from the subjacent crust and if the estimate of 1 μW m^{-3} for heat generation in the Archaean continental crust is reasonable, this portion would be 20–30 mW m^{-2}.

Lower estimates of q_* would be obtained if it could be shown that exhumation from more than 40 km was responsible for the generation of the P–T data under discussion. Table 1 shows that q_* of 40–60 mW m^{-2} is required for the conditions described above if the rocks originated at 60 km rather than 40 km. Such rocks would follow the kind of P–T–t path of Fig. 1a and would perhaps show a record of progressive metamorphism during the early part of the path, when decompression was nearly isothermal.

Table 1 Values of q_* required to sustain temperatures of 900 °C at 40 and 60 km for different values of K and q_A (see text)

q_A (mW m^{-2})	Depth of burial 40 km			Depth of burial 60 km		
	Conductivity (Wm^{-1} K^{-1})					
Heat generation distributed uniformly	2	4	6	2	4	6
0	45	90	135	30	60	90
20	35	80	125	20	50	80
40	25	70	115	10	40	70
60	15	60	105	—	30	60
80	5	50	95	—	20	50
Heat generation distributed exponentially	2	4	6	2	4	6
0	45	90	135	30	60	90
20	40	85	130	27	57	87
40	35	80	125	23	53	83
60	30	75	120	20	50	80
80	25	70	115	17	47	77

These lower estimates of q_* would result in temperatures of 400–700 °C at the base of 35 km thick crust after the overburden was removed.

In conclusion, P–T data from high grade Archaean terrains represent temperatures reached during the exhumation of the rocks from an overthick crust and are probably not lower than equilibrium profiles for this thickened crust. At present the best upper limit which may be placed on the heat flow from the Archaean mantle from these data is 2–3 times present-day sub-continental values, and in this respect the P–T data offer no improvement in constraints of Archaean geothermal regimes over those from general observations of continental integrity in the Archaean: if a craton was formed of rocks of low radiogenic heat production and high conductivity, the base of its crust need not have approached melting temperatures even with mantle heat flow of 90 mW m^{-2}. On the other hand, mantle heat flow this high is not demanded by the observations; the P–T data could well represent a thermal regime in which the heat flow from below the continental crust was 40 mW m^{-2} or less.

It would be possible to lower the upper bound on Archaean continental geothermal gradients if reliable information were obtained on the distribution of heat-producing elements in the Archaean crust and on the P–T-time paths followed during the exhumation of high grade Archaean rocks.

The lower geothermal gradients permitted by the P–T data would be consistent with the view of Archaean tectonics in which the continental thermal regime was similar to that of present day and most of the additional heat was lost from the Earth's interior by a faster rate of creation of oceanic lithosphere[1,6].

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Uranium series dating of travertine from archaeological sites, Nahal Zin, Israel

AT present, absolute dates of occupation can readily be determined for prehistoric sites no older than about 40,000 yr BP using conventional ^{14}C techniques. Ages of older sites can be obtained with the K–Ar method, but only where volcanic deposits are interstratified with the archaeological deposits. The majority of Middle and Lower Palaeolithic sites contain no volcanic deposits. However, many of these sites are associated with synchronously deposited beds of calcium carbonate, occurring either as tufa mounds left by now extinct springs, or as layers of travertine (speleothem) in the mouths of formerly inhabited caves. We show here that such calcareous deposits can be dated by the ^{230}Th–^{234}U method, as long as they are relatively impermeable and free of detritus; ages in the range 5,000–400,000 yr BP are obtainable with a precision of 5–10%. When the carbonate is deposited, it invariably contains traces of uranium but essentially no thorium. Its age can be obtained from the extent to which ^{230}Th has grown into radioactive equilibrium with its parent ^{234}U. The technique of radiochemical analysis is given in ref. 1. The ^{230}Th–^{234}U method has been found to be less reliable for the dating of molluscs[2]; although it has also been applied to the dating of bones[3], the method has often yielded contradictory results, probably attributable to continuous diffusion of radionuclides through the bone during burial[4]. Some permeable, very fine-grained tufas have also given poor results in earlier studies[5]; however, careful choice of dense, impermeable, coarsely crystalline travertines generally yields dates that are mutually consistent and coherent with other geochronological tests[6].

We have applied this method to the dating of travertines found in association with artefacts of Middle and Upper Palaeolithic typology from sites in Nahal Zin in the north-central Negev Highlands, Israel. These open-air sites have been described by Marks *et al.*[7]. Lying on the floor of Nahal Mor (Fig. 1) is a large block of travertine in which there occurs a layer of artefact-bearing breccia. The artefacts include a few blades, flakes and a single blade core. The block has evidently fallen from a massive deposit of travertine seen clinging to the steep wall of the canyon about 30–35 m above the present valley floor. The blade core is particularly interesting because it exhibits hard hammer technique, an absence of edge regularisation, platform faceting only on the margin adjacent to the face of the core, and the removal of sizeable blades. These artefacts and the core in particular resemble material found in level 4 of site Boker Tachtit (D 101)[8] located in an alluvial terrace ~1.8 km downstream from the travertine block. Artefacts from this site represent the transition from Middle to Upper Palaeolithic typology. Charcoal from level 1 of site D 101 gave ages of >45,500, >35,000 and 45,000±2,400 yr BP; the stratigraphy of the site suggests that there is very little difference between the ages of levels 1 and 4.

Travertine samples from above and below the breccia layer exhibit very uniform $^{234}U/^{238}U$ ratios and uranium concentrations (Table 1), suggesting deposition over a relatively short time. There is no significant difference between the ages of samples from above and below the breccia stratum; pooling all the data, we conclude that the artefacts were deposited at 46,500±2,900 yr BP. This agrees satisfactorily with the ^{14}C estimates at D 101. Another adjacent fallen block of travertine gave an age in the same range. The alluvial terrace on which site Boker Tachtit was deposited, although largely removed by down-cutting of Nahal Mor, can be traced up the canyon to a point 20–30 m below the travertine mass from which the dated blocks must have fallen. Water from this now inactive spring may have been used by the occupants of Boker Tachtit, who chanced to leave the artefacts found embedded in the travertine.

About 5 km to the south-east, near the head of the Nahal Aqev (Fig. 1), lies a dissected mound of travertine marking the site of a former spring (Fig. 2). A layer of colluvial sediment interlaminated between the two outermost layers comprising this travertine mound contains several artefacts of Levallois technique[9]. They resemble artefacts found in site Nahal Aqev (D 35), 150 m NNE from the fossil spring. The artefacts from site D 35 are ascribed to the early Mousterian[10] and resemble Copeland's[11] Levantine Mousterian phase 1. These artefacts

Table 1 Isotopic and age data for travertines from Avdat/Aqev area

Sample no.	Description	$^{234}U/^{238}U$	U (p.p.m.)	$^{230}Th/^{234}U$	$^{230}Th/^{232}Th$	Age (10^3 yr BP)
		Travertine blocks in Nahal Mor				
76NZ3-2	Below artefact layer	0.940±0.032	3.14	0.372±0.014	80.8	50.7±2.5
76NZ3-2A	Below artefact layer	0.950±0.019	3.04	0.362±0.012	44.1	49.0±2.0
76NZ3-1	Below artefact layer	0.938±0.030	3.81	—*	—	—
76NZ4B-2A	Above artefact layer; upper lamina	0.915±0.023	2.45	0.318±0.020	>1,000	41.8±3.1
76NZ4B-3	Above artefact layer; middle lamina	0.955±0.029	2.91	0.206±0.013	>1,000	25.0±1.8
76NZ4B-4a	Above artefact layer; lower lamina	1.161±0.030	4.71	0.344±0.077	>1,000	45.2±12.5
76NZB-4a	Above artefact layer; lower lamina	0.937±0.025	2.41	0.352±0.021	56.4	47.3±3.6
76NZ4A-1	Above artefact layer	1.054±0.062	1.35	0.380±0.030	9.9	45.2±5.2†
76NZ5-2	Another travertine block, 0.5 m away from NZ4	0.942±0.043	2.25	0.344±0.025	68.8	46.0±4.2
	Average omitting 76NZ4B-3	0.977±0.074	2.90±0.96			46.5±2.9
		Nahal Aqev, fossil spring‡				
76NZ6e-1	layer A	0.924±0.030	2.68	0.886±0.046	29.1±3.3	258^{+86}_{-46}
76NZ6a-1	layer B	0.896±0.031	2.27	0.851±0.073	50.3±26.5	228^{+127}_{-54}
76NZ6a-2	layer B	0.887±0.020	2.26	0.834±0.032	52.5	214^{+33}_{-25}
76NZ6a-3	layer B	0.929±0.020	1.87	—	—	—
76NZ6a-4	layer B	0.890±0.018	2.75	0.804±0.062	>1,000	191^{+5}_{-36}
	Average, layer B	0.900±0.019	2.37			211±19
76NZ6d-3	layer D	1.076±0.030	0.97	—*	—	—
76NZ6d-4	layer D	1.125±0.064	1.34	0.584±0.040	>1,000	85.2±10.0
76NZ1	layer D	1.060±0.030	2.12	0.496±0.023	>1,000	74±5
		Ein Aqev				
76NZ8	Fossil spring deposit	0.963±0.032	2.30	0.110±0.006	17.1	11.8±0.9†

Errors are 1σ, based on Poisson statistics for α-counting.

* No thorium recovered.

† Corrected for initial $^{230}Th/^{232}Th = 1.5$ at $t = 0$.

‡ See Fig. 2 for location of layers A to E.

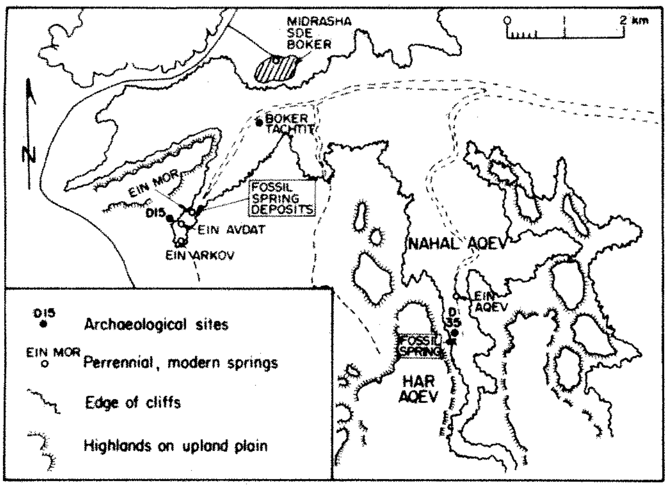

Fig. 1 Map of Nahal Zin showing locations of dated sites. Nahal Mor is the canyon in which Ein Mor and Ein Avdat are located.

also closely resemble the lithic technology of site Rosh Ein Mor (D 15), situated on the canyon rim above Nahal Mor.

The lowest layer of the travertine mound (Fig. 2A) was deposited 258,000 ± 66,000 yr BP, whereas three dates from layer B give an average age of 211,000 ± 19,000 yr BP. Layer C is undated. From layer D, two samples were obtained, one with an apparent age of 85,000 ± 10,000 yr BP (including a small correction for common ^{230}Th) and another (76NZ1) giving an age of 74,000 ± 5,000 yr BP. Unfortunately, both the artefact-bearing layer and the top layer (E) are too heavily contaminated with limestone fragments to permit dating. The artefact-bearing layer, which seems stratigraphically to be either of the same age or slightly younger than layer D, was therefore deposited at about 80,000 ± 10,000 yr BP. We infer that this was the time of occupation of site D 35, the location of which was presumably determined by the proximity of the spring[12]. The site may have been abandoned when flow from the springs ceased.

Further downstream in Nahal Aqev, the active spring of Ein Aqev is now depositing travertine on the steep walls of a box canyon. Older deposits a few metres downstream on the west side of the canyon mark earlier stages in the headward recession of the canyon. A sample from the surface of these deposits (76NZ8) shows that the spring was active 12,200 ± 700 yr BP; from the thickness of the deposits we infer that deposition started a few thousand years before that date, ~15,000 yr BP. This was close to the time of occupation of the nearby Upper Palaeolithic site D 31 (Ein Aqev), ^{14}C-dated at from 18,000 to 17,000 yr BP[13].

These results, as well as providing the first absolute date for the early Levantine Mousterian culture, demonstrate the potential of ^{230}Th/^{234}U dating of travertine in establishing an absolute time scale in archaeology.

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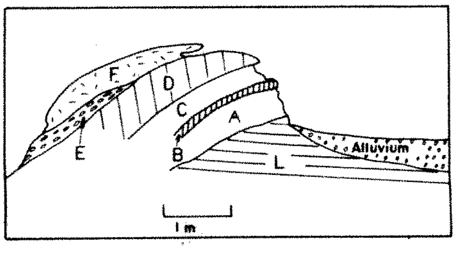

Fig. 2 Cross-section of fossil spring mound in Nahal Aqev. Layers A to D: travertine; layer E: colluvium containing Levallois flakes; layer F: travertine containing abundant limestone clasts; L: limestone bedrock.

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Phylogeny and palaeobiogeography of hadrosaurian dinosaurs

MOST geologists agree on the placement of the continents at the end of the Cretaceous. Palaeobiologists, however, hold divergent opinions[1,2]. Some workers connect South America to Africa, some connect it only to North America, while others isolate South America totally. New evidence from vertebrate palaeontology suggest that a substantial land connection between North and South America existed 85–100 Myr ago which facilitated the passage of many terrestrial vertebrates between the continents, including large herbivorous dinosaurs. Specimens of hadrosaurian dinosaurs, collected in the first half of the present century, have now been used to reanalyse not only the palaeobiogeography but also the classification and phylogeny of this group. I report here the first solid evidence of an extensive land connection for large terrestrial vertebrates of this sort between North and South America at the end of the Cretaceous.

Hadrosaurs were the most diverse and abundant late Cretaceous, terrestrial vertebrate herbivores. In most cases they seem to have comprised up to 75% of the biomass in local faunas[3], according to recovered fossil samples. Their remains can be found in every Laurasian continent and also in South America. Because most specimens are represented by post-crania alone, and most workers believe that hadrosaur post-crania are not diagnostic, it has been almost impossible to map the distribution of sub-familial taxa. It is now possible, especially with pelvic elements, to identify a genus on the basis of a single element[4]. With this new information, previously named taxa can be rediagnosed, isolated elements from all over the world can be identified, and Cretaceous hadrosaurian palaeobiogeography can be reanalysed.

An accurate, useful phylogeny must include all valid taxa from all geological ages and all localities. Previous hadrosaur phylogenies have been based on North American data only[5,6]. My new phylogeny (Fig. 1) includes all forms at the generic level and recognises two subfamilies, the Hadrosaurinae and the

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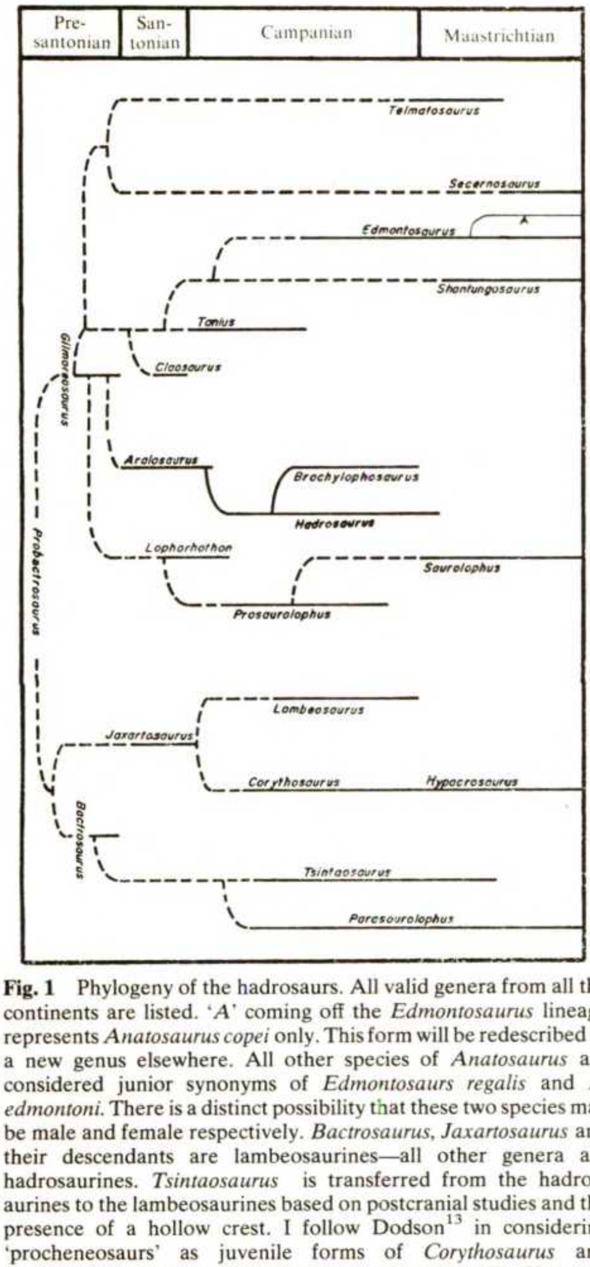

Fig. 1 Phylogeny of the hadrosaurs. All valid genera from all the continents are listed. '*A*' coming off the *Edmontosaurus* lineage represents *Anatosaurus copei* only. This form will be redescribed as a new genus elsewhere. All other species of *Anatosaurus* are considered junior synonyms of *Edmontosaurs regalis* and *E. edmontoni*. There is a distinct possibility that these two species may be male and female respectively. *Bactrosaurus*, *Jaxartosaurus* and their descendants are lambeosaurines—all other genera are hadrosaurines. *Tsintaosaurus* is transferred from the hadrosaurines to the lambeosaurines based on postcranial studies and the presence of a hollow crest. I follow Dodson[13] in considering 'procheneosaurs' as juvenile forms of *Corythosaurus* and *Lambeosaurus*. *Cheneosaurus* is certainly a juvenile *Hypacrosaurus*. *Brachylophosaurus* is transferred from the *Sauroplophus* lineage to the *Hadrosaurus* (*Kritosaurus*) lineage based on postcranial and cranial evidence.

Lambeosaurinae. The Hadrosaurinae are characterised by: relatively low sacral neural spines with a length/width ratio less than 4.5; ventrally grooved sacral centra; long gracile limb elements, non-footed ischium; prepubis with a long thin neck; ilium length/height ratio averaging 4.4; convoluted or slightly expanded pre-maxilla; long straight mandible; solid narial crests (where present). The Lamebosaurinae are characterised by: relatively high sacral neural spines with a length/width ratio greater than 4.5; ventrally ridged sacral centra; shorter and more robust limb elements; footed ischium; prepubis has a short, thick neck; ilium length/height ratio averages 5.4; greatly expanded premaxilla; shorter and anteriorly decurved mandible; hollow narial crests present. Most, if not all, genera in both subfamilies are monospecific. Evidence based on postcranial studies[4] suggests that the Saurolophinae should be included in the Hadrosaurinae.

The three pivotal genera in this report are *Secernosaurus koerneri* (new genus, new species) from Argentina, and *Gilmoreosaurus mongoliensis* (new genus, new combination) and *Bactrosaurus johnsoni*[7] both from Mongolia. All three genera display a primitive grade of organisation reminiscent of their iguanodont ancestry. *Secernosaurus* is the second most primitive hadrosaur known. It is only the second hadrosaur reported from Gondwanaland[8] and the first to be of diagnostic value (the first was by R. Casamiquela). The unique features of its ilium justify a new genus:

Class, Archosauria
Order, Ornithischia
Family, Hadrosauridae
Subfamily, Hadrosaurinae
Secernosaurus (new genus)

Holotype: FMNH P13423, two ilia, prepubis, scapula, fibula, caudals and partial braincase. Collected in 1923 by J. B. Abbott for the Field Museum of Natural History in Chicago.
Horizon: San Jorgé Formation, Rio Chico, Patagonia, Argentina.
Age: Upper Cretaceous.
Etymology: generic name from the Latin *secerno* which means to sever or divide, referring to its non-Laurasian origin.
Diagnosis: postacetabular process of ilium greatly deflected dorsomedially and elongate, unlike any hadrosaur or iguanodont. Preacetabular process of ilium deflected ventrally at an angle greater than in *Hadrosaurus* (Fig. 2). (I follow Baird and Horner[9] in recognising *Hadrosaurus* as the senior synonym of *Kritosaurus*.) Antitrochanter relatively smaller than in any hadrosaur of the same size.

Secernosaurus koerneri (new species)

Diagnosis: same as for genus.
Etymology: specific name after Dr Harold E. Koerner, professor emeritus, University of Colorado.

Gilmoreosaurus is the first representative of the Hadrosaurinae in Asia. It was originally described as a new species of the genus *Mandschurosaurus*[7] but this genus was proved to be a *nomen dubium*[4]. The species, however, contains many features that are partly iguanodont and partly hadrosaurine, hence it is appropriate to recognise this form as a valid genus:

Gilmoreosaurus (*Mandschurosaurus*) *mongoliensis*
(new genus, new combination)

Holotype: redesignated American Museum of Natural History 6551.
Horizon: Iren Debasu Formation, Cenomanian?
Diagnosis: same as original description for species[7].
Etymology: after Charles W. Gilmore, the original author.

Bactrosaurus, the first known lambeosaurine, occurs with *Gilmoreosaurus* in the same formation. The two subfamilies (Lambeosaurinae and Hadrosaurinae) are already separate at this time, which would put the origin of the family some time in the Middle Cretaceous.

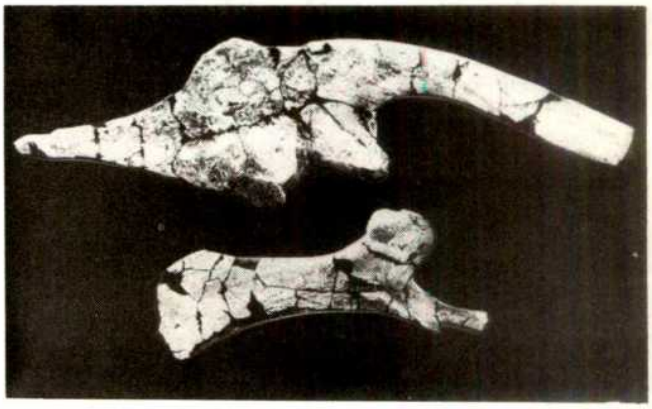

Fig. 2 *Secernosaurus koerneri* (new genus and species). Holotype FMNH P 13423. Right ilium and left prepubis in lateral view. Length of ilium between perpendiculars is 533 mm.

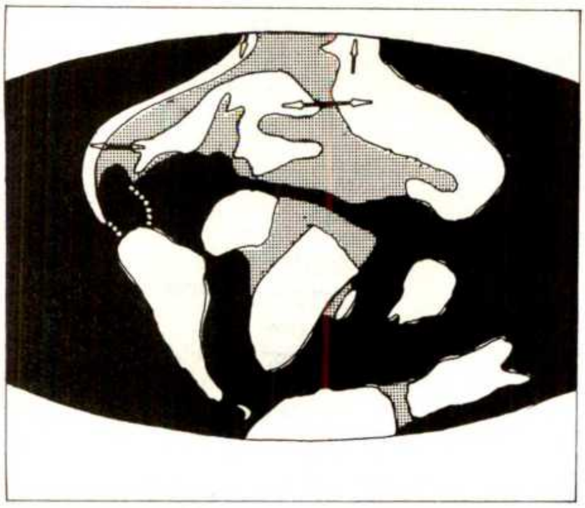

Fig. 3 Palaeogeographic reconstruction of the continents during the Turonian Transgression. Based on Tedford[2] and Smith and Briden[11].

Hadrosaurs[7,10] are considered to have originated in Asia where *Probactrosaurus*, an iguanodont and probable ancestor, has been found. The predominance of primitive hadrosaurs and advanced iguanodonts in Laurasia does not necessarily imply that the family originated there. The fossil record only indicates that by the Mid Cretaceous, hadrosaurs were present in both Laurasia and Gondwanaland, and that they had differentiated into two subfamilies. Dispersal between South America and Laurasia could have been by way of Africa or North America (Fig. 3). Most workers[2,11] believe that Africa was isolated from South America sufficiently early so as not to have been involved in the Upper Cretaceous dispersal of marsupials, so that dispersal must have occurred by way of North America. The two possible links with South America were through Panama or the Caribbean arc. Both hadrosaurs and ceratopsians[12] are found in South America and so the route used must have been a major land connection at some time because both groups consist of large terrestrial vertebrates not subject to waif dispersal or rafting. Dispersal is not considered to have occurred after the Late Cretaceous transgression, because Santonian hadrosaurs were already advanced (derived) beyond the *Secernosaurus* grade of evolution.

It is possible that the presence of hadrosaurs in Laurasia and Gondwanaland represents vicariance rather than dispersal; if so, hadrosaurs would be expected to occur on most continents. Their absence in India and Australia, where Upper Cretaceous deposits with vertebrates are known, indicates otherwise. This, however, is negative evidence and disprovable. Until hadrosaurs are found in India and Australia, the dispersal model is to be favoured.

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Kanamycin-resistant *Scenedesmus obliquus* showing simultaneous resistance to other antibiotics and polychlorinated biphenyls

WE have been studying the effects of polychlorinated biphenyls (PCBs) and DDT on several planktonic algae, and report here that we have isolated a kanamycin-resistant strain of *Scenedesmus obliquus* which shows simultaneous resistance to other antibiotics, PCBs and Cetavlon (ICI), a quaternary ammonium compound with algicidal properties[2]. This novel observation suggests a possible genetic control of sensitivity to PCBs in *Scenedesmus*.

S. obliquus (UTEX 2015) was one of the test organisms and was obtained as an axenic culture. This strain was grown mixotropically in liquid TAP (Tris–acetate–phosphate)[4] and when phototrophic growth was being tested acetate was omitted from the medium, which was then a minimal TAP (M-TAP). *S. obliquus* was able to grow on M-TAP in light, thus confirming it to be a phototrophic strain. Agar plates were prepared by adding 1.5% of agar (Difco) to the liquid medium. Required concentrations of antibiotics were filter sterilised and added just before the plates were poured. To test for sensitivity or resistance to antibiotics, 0.1 ml of a *S. obliquus* culture containing 1×10^7 cells ml^{-1} was spread on TAP- and M-TAP-containing antibiotic plates. The plates were incubated for a week in a growth cabinet at 22 °C with continuous illumination. Growth was determined visually.

The control plates without antibiotics showed a heavy growth in the form of a continuous green lawn; there was no comparable growth on antibiotic-containing plates. Thus, we concluded that *S. obliquus* was sensitive to kanamycin (100 μg ml^{-1}), chloramphenicol (50 μg ml^{-1}), streptomycin (300 μg ml^{-1}) and tetracycline (50 μg ml^{-1}). However, when these plates were held at 22 °C for 1 month, we found 7–8 discrete, actively growing colonies on each of the kanamycin-treated plates only. These

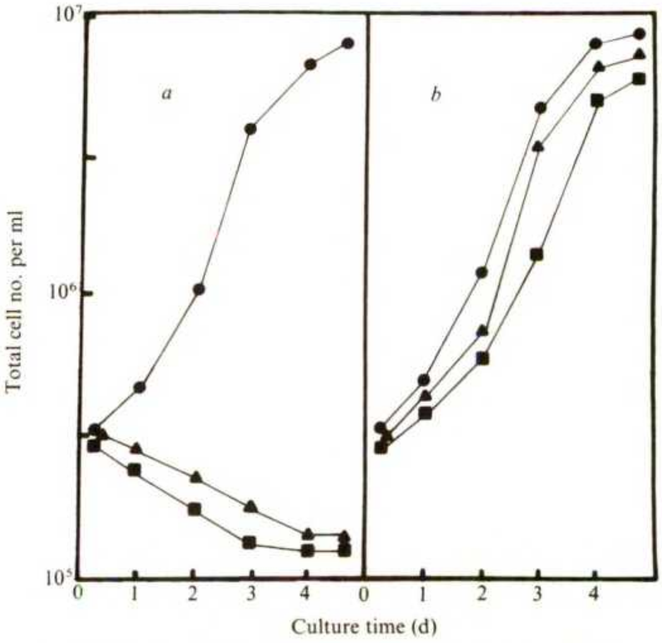

Fig. 1 Effects of PCBs (Aroclor 1242) and Cetavlon on the growth of *S. obliquus* (*a*) and kanamycin-resistant *S. obliquus* (*b*). Cells were grown in liquid TAP and total cell number represents viable cell counts within a 90% confidence level. ●, control; ▲, 5 p.p.m. Aroclor 1242; ■, 5 p.p.m. Cetavlon.

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colonies were isolated and named 2015-kr (kanamycin resistant) cells of *S. obliquus*, and cells from each colony were tested separately on TAP plates containing various antibiotics. The progeny of each colony showed similar results and so were treated as a single resistant strain for further experiments. When these presumptive mutants were tested on kanamycin, streptomycin, chloramphenicol and tetracycline plates, they all showed simultaneous resistance on TAP plates. The property of simultaneous resistance had become a permanent feature; this was confirmed by repeated tests on antibiotic-containing plates. Surprisingly, 2015-kr cells failed to grow when plated on TAP media without acetate (M-TAP) supplied with the antibiotics, although the original strain could grow equally well on M-TAP or TAP.

Examination under the optical microscope showed these cells (2015-kr) to be smaller and more round than the parental strain, and lacking coenobia, characteristic of *Scenedesmus*. However, to prove that these were a true derivative of *Scenedesmus* and not a contaminant of another alga, we grew this strain on several different media including some which were unfavourable for active growth of *S. obliquus*. In one such medium, proteose agar, the resistant cells did not show active growth but did produce typical *Scenedesmus* morphology[3]. The identity of these cells was then independently confirmed as *Scenedesmus*.

Previously, *Scenedesmus* (2015) was found to be sensitive to PCB at 0.25 p.p.m. and to an algicide, Cetavlon, at the 1 p.p.m. level. When the kanamycin-resistant variety was tested on these chemicals it was found to be resistant above the 5 p.p.m. level, a concentration lethal for the parent strain (Fig. 1).

The fact that 2015-kr cannot grow on the M-TAP containing antibiotics suggests that the genes affected here are possibly chloroplastic. However, both the parental strain and 2015-kr could grow on M-TAP without antibiotics; this indicates that *S. obliquus* was phototrophic whereas 2015-kr behaved as an obligate heterotroph in the presence of antibiotics. Extensive work on the inheritance of antibiotic resistance involving chloroplastic genes has been carried out in *Chlamydomonas reinhardtii*[1,9–12], where it was shown that many of the mutations conferring antibiotic resistance were present in clusters in chloroplastic DNA. Streptomycin has been used as a selective agent to isolate resistant strains to other antibiotics in *C. reinhardtii* and these mutations were also mapped as chloroplast mutations[1]. However, *C. reinhardtii* possesses a sexual cycle which enables easy differentiation between nuclear and cytoplasmic genes, whereas *Scenedesmus* reproduces asexually and so it is not possible to carry out genetic crosses. Although there is one report of sexuality[8] in *Scenedesmus*, it has not yet been confirmed.

Our previous studies on *Chlorella* and *Spirodella*[5–7] in relation to their sensitivity to PCBs showed that photosynthesis was inhibited by these chemicals. Sensitivity to PCBs could be decreased by addition of a supplementary carbon source to the medium which allowed the cells to by-pass the inhibitory effects of PCBs (in preparation). We believe this to be the first report of an alga, previously sensitive to PCBs and several antibiotics known to affect chloroplast ribosome synthesis, which has acquired simultaneous resistance to all these chemicals. This suggests that, for *Scenedesmus* at least, responses to environmental pollutants such as PCBs can be controlled by specific genes similar to those conferring antibiotic sensitivity. These genes seem to be located in the chloroplast, but the possibility of their being nuclear genes concerned with the function of cytoplasmic organelles is not ruled out.

More recently, we have been able to repeat the isolation of kanamycin-resistant *Scenedesmus* cells; *S. obliquus* will apparently produce approximately one resistant cell per 5×10^5 cells, but this mutation only occurs on kanamycin plates when grown over a period of 1 month. These new isolates are under further investigation.

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Ciliate protozoans as links in freshwater planktonic food chains

THE potential importance of bacteria in freshwater planktonic food webs is recognised[1], but the mechanisms of their incorporation are not fully known. Most planktonic bacteria are not attached to particles and are less than $1\ \mu m$ (refs 2–4) in size—a range inefficiently filtered by crustacean zooplankton[5]. *Daphnia* and *Diaptomus* feed at lower rates on free living bacteria than on algae[6,7]. Bacterial food sources have been used with varying degrees of success to culture zooplankton[8–11]. These variations may be due to the unnatural range of concentrations used and the uncontrolled effects of different culture media, laboratory strains and the degree of accessibility to the zooplankton (because of cell clumping and sedimentation). Ciliate protozoan microzooplankton may be important intermediaries which transform ultrafine organic matter, including bacteria, into a particle size range readily available to crustacean zooplankton. Here we present evidence that ciliates are cropped by crustacean zooplankton in the natural environment. We have also measured feeding, filtering, and assimilation rates of *Daphnia magna* on both a large and small ciliate in the laboratory and have observed feeding behaviour directly. Dissolved organic carbon, bacteria, ciliate protozoans and crustacean zooplankton may represent a significant pathway of carbon flow in the freshwater plankton.

Ciliate protozoa ingest bacteria in nature[12] and grow on them as a sole food source in the laboratory[13]. Furthermore, field studies indicate that planktonic ciliates are actively preyed on in nature. Manipulations of crustacean zooplankton densities in enclosures of lake water[14] reveal that ciliates, as well as algae, are reduced in abundance when crustacean zooplankton densities increase (Table 1). In these field studies, remains of ciliates were found in the feeding appendages and gut contents of the copepod *Cyclops scutifer*. Other microzooplankton, the rotifers, were unaffected by manipulations of crustacea, indicating that they are not major links in this system. Similar reductions in protozoan numbers in the presence of marine copepods occurred in laboratory studies[15].

Feeding studies of *Daphnia magna* were made using the large ciliate *Paramecium caudatum* ($70\times200\ \mu m$; volume $6.15\times10^6\ \mu m^3$) and the small ciliate *Cyclidium glaucoma* ($20\times30\ \mu m$; volume $6.54\times10^4\ \mu m^3$). Protozoans were raised on suspensions of *Enterobacter aerogenese*. Bacteria were grown for 24 h at 38 °C in defined medium, and resuspended in Cerophyll to a final concentration of 1×10^6 cells ml^{-1}. Protozoans were saturation labelled by incubating in this suspension for 7 d at 24 °C. They were separated from the bacteria by geotaxis[16] and

Table 1 Effect of manipulations of crustacean zooplankton on algae and microzooplankton

	Levels of crustacean abundance*				
	Absent	Natural	Enriched	χ^2	P
Ciliates	35.0	25.0	12.0	22.167	<0.001
Total algae	35.0	24.0	13.0	20.167	<0.001
Rotifers†	24.5	33.5	18.0	1.792	NS

**Daphnia galeata mendotae*, *Diaptomus minutus*, *Cyclops scutifer*, and *Epishura lacustris* were removed by filtration or enriched 8-fold in 0.5 m^3 enclosures of lake water on 12 occasions throughout the year[14]. Whole water was collected from the enclosures after 4 d, fixed with Lugol's iodide preservative and counted with an inverted microscope. Because of seasonal variations in abundance, data are reported as sums of ranks. Actual ciliate counts from natural epilimnetic lake water ranged from 0.3 ml^{-1} to 9.0 ml^{-1}. Ranks were summed for each level over the 12 dates and used to compute a Friedman χ^2 with a critical value of 6.167 ($P<0.05$). NS, not significant.

†*Keratella*, *Kellicottia* and *Conochilus*.

resuspended in filtered (0.45 μm Millipore), autoclaved aquarium water in which the experimental *D. magna* were raised. Protozoans were sized, counted in a Sedgewick–Rafter cell and diluted to a final concentration of 135 individuals ml^{-1} (0.985 μg dry weight ml^{-1}) for *P. caudatum* and 2.55×10^3 individuals ml^{-1} (0.823 μg dry weight ml^{-1}) for *C. glaucoma*. Both concentrations are above the incipient limiting concentration for *D. magna* and thus feeding rates were maximised.

Observations of *D. magna* feeding on individual *P. caudatum* were made using an inverted microscope (Zeiss) with 10× eyepieces and a 63-mm Luminar objective permitting a working distance of 6.0 cm and a 2.0-mm depth of field. Live *D. magna* were suspended in a large volume feeding chamber (5×5×5 cm) by attaching the head shield to a fine glass rod tipped with silicone grease. This is an improvement over previous methods of observing compressed *Daphnia*, as it allows free circulation of feeding currents and unobstructed movements of the abdomen, swimming appendages and carapace.

The bacterium *Enterobacter aerogenes* assimilated the ^{14}C-labelled glucose and served as a food source for the protozoans which incorporated the label. *D. magna*, in turn, assimilated this carbon from the protozoans it ingested. Filtering rates of *D. magna* fed on either protozoan showed a positive linear relationship to body size (Fig. 1). *D. magna* ingestion, filtering, and assimilation rates were higher when fed the smaller protozoan, *C. glaucoma*, which is in the size range of many algae, than when fed the large *P. caudatum* (Table 2). Ingestion and filtering rates on both protozoa were lower than rates measured in similar conditions on algae, although the assimilation efficiencies were comparable[17].

Table 2 Mean ingestion rates, assimilation rates, and assimilation efficiencies for *Daphnia magna* fed on *Paramecium caudatum* and *Cyclidium glaucoma*

Food organism	Ingestion rate* $\bar{x}\pm$s.e.m.	N	Assimilation rate* $\bar{x}\pm$s.e.m.	N	Assimilation efficiency
P. caudatum	0.1785±0.0335	5	0.1166±0.0103	4	65%
C. glaucoma	0.4793±0.0110	5	0.3161±0.0303	4	66%

Ingestion and assimilation rates were measured by adding 25–35 *D. magna* to replicate 300 ml BOD bottles containing protozoan suspensions and measuring radioactive uptake in 15 min and incorporation during 1 h (ref. 17). *D. magna* of comparable body sizes were used to measure ingestion rates and assimilation rates for the two species of protozoa. Assimilation efficiencies are calculated as the per cent of the material ingested that is assimilated.

*Ingestion rates ($t=2.608$, $P<0.025$) and assimilation rates ($t=6.232$, $P<0.001$) were significantly different for the two protozoa.

Rates are expressed as μg dry weight of food per animal per hour.

Abbreviations: $\bar{x}$, mean; s.e.m. standard error; N, No. of replicates.

Observations of *Daphnia magna* feeding on *P. caudatum* indicated that only some of the protozoans that entered the filtering current were brought inside the carapace and into the food groove. These were broken up by the movement of the thoracic appendages, labrum, and mandibles and ingested with some loss of material. This slovenly feeding in *Daphnia* is analogous to loss of food material in the mouth parts of other planktonic crustacea[18]. This confirms the interpretation of observations made of animals feeding on large or elaborately shaped algal cells and colonies[19]; food particles, with dimensions larger than the maximum size of rigid spherical particle (38 μm for *D. magna*)[20] that can be ingested whole, can be manipulated and ingested but with reduced efficiency. Measured filtering rates of these particles will be less than the actual rate at which water passes through the filtering apparatus because of rejection of particles that clog the filtration apparatus and loss of material broken up during filtration. The upper and lower limits to the particle size ranges available to *Daphnia* are thus not absolute. Rather, there is a decrease in handling efficiency as particles move away from an optimal size range. Optima for different age classes and species may be the mechanisms by which zooplankton divide up food resources. Behavioural changes may allow individuals to shift optima to accommodate different food particle spectra.

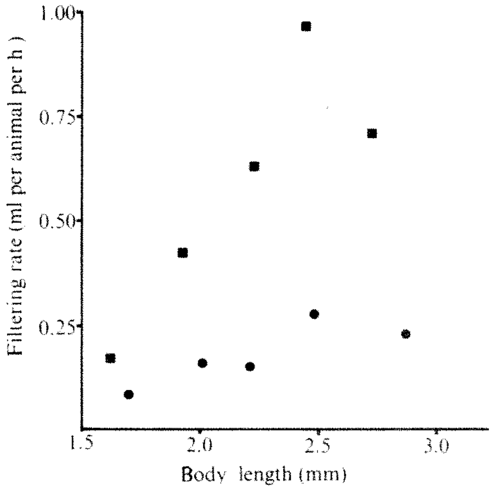

Fig. 1 Filtering rates of *Daphnia magna* on the small ciliate (20×30 μm, *Cyclidium glaucoma* (■), and the large ciliate (70×200 μm), *Paramecium caudatum* (●).

Studies of ciliates feeding on bacteria suggest that a minimum concentration of approximately 10^6 bacteria ml^{-1} is necessary for growth and reproduction of protozoans[21,22]. Nominal concentrations of bacteria in lakes range from 10^5 to 10^6 ml^{-1} (ref. 2). During the declining phase of algal blooms, however, bacterial production is elevated and high concentrations of ciliates (10^2 ml^{-1}) have been observed[23]. Protozoans may also be concentrated in zones near the metalimnetic boundaries, at chemoclines, and above the sediments[24]. If these organisms are cropped by larger zooplankton, as our field and laboratory experiments suggest, then they represent, at least temporally, an important mechanism by which DOM, bacteria, and ultrafine detritus enter planktonic food chains.

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Low levels of fixed nitrogen required for isolation of free-living N_2-fixing organisms from rice roots

ISOLATION and counting of N_2-fixing bacteria from the natural environment have until now been conducted on selective N-free media. It is likely that some N_2-fixing bacteria are overlooked by using this procedure. We now report that most of the bacteria present in rice roots are N_2-fixers, but that they require a supply of mineral or organic N for growth. In the presence of organic nitrogen, nitrogenase activity is detected in these organisms. The requirement for organic nitrogen for the detection of nitrogenase is also true for N_2-fixing cultures of free-living rhizobia[1–3].

Rice plants (IR26) were dug out from an unfertilised wetland rice field and the roots were washed to remove soil. The washed roots were cut into segments and shaken vigorously with glass beads to remove rhizoplane bacteria. After several washings the root fragments were macerated to provide a suspension of 'inner rhizoplane' bacteria. The lower portion of the stem was also blended to make a suspension of bacteria living in or on the stem. Suspensions were made in sterile tap water and diluted without exclusion of oxygen. Diluted suspensions were spread on tryptic soy agar (0.1% Difco tryptic soy broth and 1.5% Noble agar), a medium used for counting aerobic heterotrophs[4]. The population of heterotrophic aerobic bacteria per g fresh weight sample was 2.3×10^7 organisms for rhizosphere soil, 1.2×10^7 for stem, 1.5×10^7 for rhizoplane, and 1.1×10^8 for inner rhizoplane.

Colonies from each fraction were selected and tested for nitrogenase activity using the acetylene reduction assay. Acetylene reduction activity (ARA) was measured for a 24-h period after 3 days growth at 35 °C on semi-solid glucose yeast extract medium (in $g\,l^{-1}$: 5.0 glucose, 0.1 Difco yeast extract, 0.5 K_2HPO_4, 0.04 $FeSO_4 \cdot 7H_2O$, 0.2 $MgSO_4 \cdot 7H_2O$, 0.0059 $NaMoO_4 \cdot 2H_2O$, 0.2 $CaCl_2 \cdot 2H_2O$; in $mg\,l^{-1}$: 0.15 H_3BO_3, 0.11 $ZnSO_4 \cdot 7H_2O$, 0.07 $CoSO_4 \cdot 7H_2O$, 0.005 $CuSO_4 \cdot 7H_2O$, 0.004 $MnCl_2 \cdot 4H_2O$, *p*H 7.0, and Difco Noble agar 1.75 $g\,l^{-1}$). The percentage of bacterial isolates proving nitrogenase positive (more than 6 nmol C_2H_4 per tube) was 81% (95/117) for inner rhizoplane bacteria, 76% (19/25) for rhizoplane bacteria, 37.5% (9/24) for bacteria from the stem, and 2.4% (1/41) for rhizosphere soil bacteria.

Because of the high percentage of inner rhizoplane isolates showing nitrogenase activity, the nutritional requirement of bacteria of the most predominant colony type (about 90% of nitrogenase positive isolates) was studied further. None of the isolates could grow on nitrogen-free basal medium (glucose + mineral salts). The majority (31 of 40) grew on basal + ammonium medium (NH_4Cl 61 $mg\,l^{-1}$), but the growth was much poorer than on basal medium supplemented with casamino acids (vitamin-free, Nutritional Biochemicals) or yeast extract (0.1 $g\,l^{-1}$).

For isolates that grew best on basal medium plus casamino acids, nitrogenase activities were measured after 3 days of growth on various semi-solid media (Table 1). Activities were high in the presence of casamino acids but a vitamin mixture only slightly stimulated activity. No activity was detected in N-free medium or ammonium-supplemented medium. Each amino acid was tested individually and all could support growth and nitrogenase actvitity.

Table 1 Acetylene-reducing activity of inner rhizoplane isolates in semi-solid media

Medium	C_2H_4 produced (nmol per tube per day)
Expt 1 (28 isolates tested)	
Basal (glucose + mineral salts)	0
Basal + yeast extract (0.1 $g\,l^{-1}$)	97 ± 11
Basal + NH_4Cl (16 mg N l^{-1})	0
Basal + casamino acid (0.1 $g\,l^{-1}$)	96 ± 18
Basal + glutamic acid (0.1 $g\,l^{-1}$)	39 ± 8
Expt 2 (29 isolates tested)	
Basal + yeast extract	219 ± 17
Basal + casamino acid	144 ± 13
Basal + glutamic acid	120 ± 22
Basal + yeast extract + succinic acid (10 mM)	202 ± 14
Expt 3 (29 isolates tested)	
Basal + casamino acid	222 ± 7
Basal + vitamin mixture*	58 ± 4
Basal + vitamin mixture + casamino acid	251 ± 13

*Vitamin mixture: vitamin B12, 0.015 μM; *p*-aminobenzoic acid, nicotinic acid, biotin, riboflavin, pyrodoxine, folic acid, and Ca-pantothenate, all 0.8 μM.

The extent of inhibition of nitrogenase activity in the presence of oxygen was determined. When initial p_{O_2} was 0.01 atm, nitrogenase activity was maximal while at p_{O_2} 0.2 atm and 0.05 atm, nitrogenase activity was not detected. In anaerobic ($p_{O_2} < 0.001$) culture, slight activity of nitrogenase was detected. Like other aerobic N_2-fixing bacteria, such as *Azospirillum*, these bacteria require microaerophilic conditions for the expression of nitrogenase.

To confirm N_2-fixing activity a gas mixture composed of 20% $^{15}N_2$ (98 atom % excess), 1% O_2, 79% Ar was introduced into 4-day static bacterial cultures on basal medium plus glutamate (0.1 $g\,l^{-1}$) and grown for the following 24 h at 35 °C. The two isolates used for the analysis incorporated 0.18–0.25 atom % excess into cellular nitrogen. *Azospirillum lipoferum* (SpBr17), which was used as a positive check strain, showed a higher rate of incorporation (1.16 atom % excess). Excretion of fixed nitrogen into the medium was negligible in all cases.

Nitrogenase-positive isolates from the inner rhizoplane were all small rods (0.5×2.6 μm), Gram-negative, catalase and oxidase positive, lacked cellular motility, and produced acid from lactose, sucrose, and glucose without gas formation and reduced nitrate. Thus these isolates resemble *Achromobacter*[5,6]. The only known N_2-fixing isolate of this genus was reclassified as *Klebsiella pneumoniae*[7].

A possible explanation for the inability of the isolates to grow on nitrogen-free media is that the nitrogenase activity (as measured by ^{15}N experiments) is too low. A low oxygen concentration produced in the presence of greater cell mass might be more favourable for nitrogenase production, however. The

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oxygen factor is another possible explanation of the growth characteristics. Free-living N_2-fixing rhizobia[8] are unable to grow on N-free media because they cannot assimilate fixed nitrogen: this is probably not the case for our root isolates as excretion of fixed nitrogen into the medium was negligible. We have not yet studied the role of amino acids in nitrogenase induction.

The use of nitrogen-containing media for the isolation of nitrogenase-positive bacteria could reveal a much higher incidence of nitrogenase genes in prokaryotes than was previously suspected.

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Postnatal cerebellar cells of *staggerer* mutant mice express immature components on their surface

THE histogenesis of the central nervous system is controlled, in part, by a defined sequence of cell-surface interactions[1,2]. Cell-surface carbohydrates are prominent constituents of the external faces of plasma membranes[3–5] and are thought to be important to biological recognition[6,7]. Recently, it has also been shown that some cell-surface carbohydrates change during the first 2 d after birth, an important period in development of the mouse cerebellum[8,9]. Certain neurological mutations have been shown to express defects in positioning of particular cell types at various stages during cerebellar histogenesis[2,11–13]. One of the most interesting of these is the *staggerer* (*sg*) mutant[14]. In this disorder granule cells degenerate after they have migrated into position in the granular layer. Before this expression of the disorder, the sites of synapse formation between the parallel fibre axons of granule cells and the dendrites of Purkinje cell neurones fail to form; in fact, even the tertiary branchlet spines, the potential postsynaptic sites, do not form[15–19]. Even at birth, before obvious expression of the Purkinje cell defect, external granule cells of *sg* exhibit a reduced proliferative rate which results in a reduced number of external granule cells[20].

In this report, antibodies specifically directed against microbial polysaccharides have been applied as carbohydrate probes to developing cerebellar cell populations *in vitro*. Anti-meningococcus type-B polysaccharide antibodies (anti-B) (antigenic determinant neuraminic acid) preferentially react with pre- and neonatal cerebellar cells of C57BL/6J mice, but do not react with 7-d-old (P7) cells. Anti-α-1,6-mannan antibodies, on the other hand, react with both prenatal and postnatal cerebellar cells. Comparable results have been obtained with lectins as surface carbohydrate probes[10]. Agglutination of cerebellar cells with concanavalin A and wheatgerm agglutinin decreased from embryonic day 13 (E13) to postnatal day 7 (P7). Using anti-B and anti-α-1,6-mannan antibodies to determine the distribution of their respective antigens on the surface of +/+ and *sg/sg* cerebellar cells *in vitro*, we found that P7 *sg/sg* cell cultures express immature cell-surface carbohydrates.

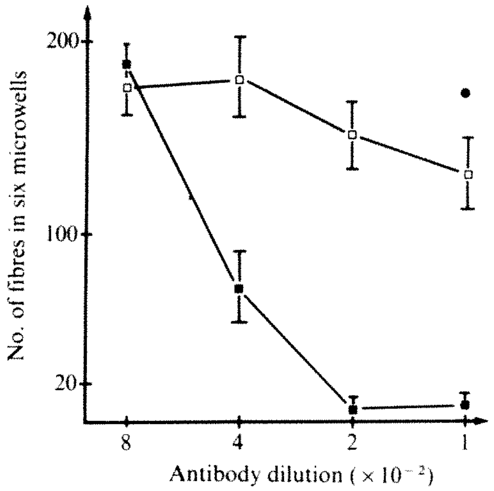

Fig. 1 Inhibition of fibre formation in microwell cultures of 7-d-old *sg/sg* cerebellum in the presence of various dilutions of anti-meningococcus type-B antibodies (anti-sialic acid) (■). The inhibition was overcome when antibodies were incubated overnight with 10 µg of meningococcus type-B polysaccharide before adding the antigen–antibody complex to the culture (□). Normal rabbit serum did not inhibit fibre formation (●). The numbers plotted represent the mean of three experiments of the total number of fibres in six microwells.

The present work is one step in a study to determine whether the defect in some of these neurological mutants might be in cell-surface constituents and whether their alterations might affect the sequence of cell–cell interactions required to generate functional connections in the developing cerebellum.

A microwell tissue culture system, in which cells show certain behaviours reminiscent of *in vivo* cerebellar development[21], was used to determine anti-carbohydrate antibody reactivity. This system allows the assay of differential cell assembly, migration and fibre outgrowth. The large number of very reproducible

Table 1 Fibre outgrowth in microwell cultures of 7-d-old *sg/sg* and +/+ cerebellum in the presence of anti-carbohydrate antibodies

			No. of fibres in 6 microwells			
Expt	Antibody	Dilution	+/+	% Inhibition	*sg/sg*	% Inhibition
1	Rabbit serum	1:50	180		193	
2			178		169	
3			222		176	
1	Anti-B	1:200	162	10	16	92
2			148	17	12	93
3			195	12	38	78
1	Anti-α-1,6-mannan	1:300	14	92	129	33
2			2	99	118	30
3			8	96	108	39

A single-cell suspension (1×10^7 cells ml^{-1}) of 7-d-old +/+ and *sg/sg* cerebellum, respectively, were plated at 5 µl per microwell, and 5 µl of antibody dilution in medium then added. Fibre formation was scored in six microwells after 2 d *in vitro* using a phase contrast light microscope. The variation among cultures was determined as ±20%. The data represent the total number of fibres in six microwells obtained in three different experiments. The number of fibres obtained in cultures containing rabbit serum were used as standard for 100% fibre formation per experiment.

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cultures obtained from each cerebellum provides a statistically suitable assay system for study of the functional effect of reagents that bind to specific cell-surface components. The number of fibre cables formed between cell aggregates was determined in the present study as a quantitative assay for antibody reactivity.

sg/sg Mutant mice were obtained on an inbred background (C57BL; $^{+}dse/sg^{++}$) as bred in our own colony. P7 cerebella of *sg/sg* and +/+ (*dse/dse*) were removed and dissociated into single cells as described elsewhere[21]. Single-cell suspensions contained $2–3 \times 10^6$ (*sg/sg*) and $1–2 \times 10^7$ (+/+) cells per cerebellum. Cells were cultured in basal Eagle's medium, supplemented with 10% horse serum (Gibco) at a concentration of 5×10^4 cells per 10 μl per microwell.

Table 2 Results of radioimmunoassay to determine cell-surface carbohydrate patterns in +/+ and *sg/sg* cerebellum

Antibody	Dilution	Radioactivity (c.p.m.) +/+	*sg/sg*
Rabbit serum	1:50	20±5	15±7
Anti-B	1:200	50±30	16,000±1,200
Anti-B + 10 μg meningococcus type-B polysaccharide	1:100	65±15	220±65
Anti-α-1,6-mannan	1:300	12,000±300	2,550±275
Anti-α-1,6-mannan + 40 μg Δ1,6-mannan	1:100	70±25	110±35

P7 +/+ and *sg/sg* cerebellar cells were cultured as described in the legend to Table 1. After 16 h *in vitro*, cultures were washed once in ice-cold phosphate-buffered saline (PBS). 5 μg of ^{125}I-labelled purified goat anti-rabbit IgG antibodies (~8.5×10^6 c.p.m. per 14.5 mg goat IgG per ml) were added and cultures incubated at 4 °C. After 2 h cultures were washed twice in 4 °C PBS and once in 10% trichloroacetic acid. The tissue was then removed from the microwell and assayed for counts. Hapten inhibition was carried out as described in the legend to Fig. 1.

The pattern of cell reorganisation in the microwell cultures into cell reaggregates and connecting fibre bundles was identical in *sg/sg* and +/+ cultures[21]. This pattern of *in vitro* cell reorganisation was not unexpected, as *in vivo* histogenesis of *sg* cerebellum is not markedly perturbed as early as P7. Three assay systems were applied to characterise alterations of cell-surface determinants on *sg/sg* cerebellar cells in microwell cultures.

First, the results in Table 1 demonstrate fibre formation in +/+ and *sg/sg* cerebellar microwell culture in the presence of anti-α-1,6-mannan and anti-B antibodies. (Previous studies have shown that these antibodies do not kill living cells[8,9].) Fibre formation in P7 +/+ cell cultures was inhibited 95% with a 1:300 dilution of α-1,6-mannan antibodies, whereas the same antibody concentration inhibited only ~33% of fibres in *sg/sg* cultures. On the other hand, anti-B had little effect on P7 +/+ cultures (~15%), but inhibited fibre formation in *sg/sg* cultures up to 93%. The antibody reaction was polysaccharide specific, as 10 μg ml^{-1} purified type-B polysaccharide inhibited the reaction (Fig. 1), whereas polysaccharides of other specificities (dextran, pneumococcus type C carbohydrate, α-1,6-mannan) had no effect (not shown). Anti-α-1,6-mannan antibody reactivity, on the other hand, was abolished after preincubation of antibodies with yeast *Saccharomyces cerevisiae* mutant X2180-1A-5.

As discussed above, anti-B antibodies block fibre outgrowth in prenatal and neonatal but not P7 cell populations cultured in microwells[8,9]. The inhibition by anti-B antibodies of fibre outgrowth in *sg/sg* cell cultures suggests, therefore, that 7-d-old *sg/sg* cerebellar cells express immature cell-surface carbohydrates. Although cultures are unsuccessful with cerebellum from animals older than P7, preliminary immunohistological data show corresponding cell-surface alterations in cryostat sections of *sg/sg* cerebellum as late as P18 (ref. 22).

In addition to the expression of embryonic cell-surface determinants, alteration of surface characteristics are indicated. As shown earlier[8], anti-α-1,6-mannan antibodies did not discriminate between cells of different age in microcultures of +/+ but inhibited only ~30% of fibre formation in *sg/sg* cultures. The cell-surface determinants expressed on *sg/sg* cerebellar cells *in vitro* and *in vivo* resemble, therefore, not only immature surface antigens (as recognised by anti-B antibodies) but also constituents which seem to be structurally different from those expressed on +/+, as indicated by the loss of anti-α-1,6-mannan-specific antibody-binding sites.

Alterations of cell-surface carbohydrate pattern were detected also by a radioimmune assay using purified ^{125}I-labelled goat anti-rabbit IgG antibodies to detect the anti-carbohydrate antibodies bound to the surface of either +/+ or *sg/sg* cerebellar cells *in vitro*. The results shown in Table 2 confirmed those obtained from fibre outgrowth inhibition studies, in that alteration of cell-surface determinants on *sg/sg* cerebellar cells are in part due to alterations in cell surface carbohydrate pattern accessible to antibody recognition.

Further evidence for the expression of embryonic cell-surface characteristics on postnatal *sg/sg* cells has been obtained by studies of the effect of epidermal growth factor (EGF) on P7 *sg/sg* and +/+ cells cultured in microwells. As discussed elsewhere[23], EGF stimulates E16–P1 astro- and oligodendroglial cells to migrate out of reaggregates and to proliferate, but is without effect on cells older than P1. P7 *sg/sg* cells, however, respond to EGF at doses comparable to those required for E16–P1 +/+ cell response (1.5 μg ml^{-1}) by an increased migration and proliferation of glial cells.

Further studies will be required to elucidate the significance of the expression of immature and/or altered cell-surface molecules to the *sg/sg* mutation, and, in particular, to determine the role of cell-surface carbohydrate in the abnormal cerebellar development of *sg/sg*.

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Destruction of inferior olive induces rapid depression in synaptic action of cerebellar Purkinje cells

THE climbing fibre afferents (CFAs) are a structure unique to the cerebellar cortex; they originate, presumably solely, from the inferior olive (IO) and make an extensive, excitatory synaptic contact with dendrites of cerebellar Purkinje cells (P cells)[1]. The importance of the CFAs in cerebellar functions has been emphasised in connection with the learning process which may occur in the cerebellar cortex[2,3], and also in connection with the development and maintenance of normal dendritic structures of P cells[4–7]. An interesting recent finding is that after destruction of the IO electrical stimulation of the cerebellar cortex fails to elicit the normal inhibitory synaptic action of P cells on their target neurones in vestibular nuclei[8,9]. This not only urges reinterpretation of the symptoms arising from destruction of the IO[10,11], but also raises a basic general problem of remote interaction in neuronal networks. Here, we report that the depression of the P-cell action develops unexpectedly fast following destruction of the IO, and also present evidence indicating that this remote effect is mediated largely by a non-impulse transmission process, presumably an axonal flow, in CFAs and P-cell axons.

The vestibulospinal reflex system was chosen as the experimental material. The lateral vestibulospinal tract cells (LVST cells), located in the nucleus of Deiters, receive monosynaptic excitation from primary vesibular afferents[12] and monosynaptic inhibition from P cells in the lateral zone of the vermis of the cerebellar anterior lobe[1] (Fig. 1*a*). These P cells in turn receive CFAs from the caudal portion of the dorsal accessory olive (CDAO)[13]. Sixteen adult albino rabbits were anaesthetised by α-chloralose plus urethane (60 and 400 mg per kg, respectively), immobilised by gallamine triethiodide, and artificially respired. The dorsal surface of the lower medulla was exposed by opening the atlanto-occipital membrane. A part of the IO was destroyed electrolytically by passage of 0.5 or 1.0 mA d.c. currents for 1 min through a metal electrode (Fig. 1*a*). Location of the metal electrode in the IO was monitored by observing effects of stimulation through it in evoking field potentials in the nucleus of Deiters or in the vermal cortex of the cerebellum, and was later confirmed histologically.

Synchronised reflex discharges of LVST cells evoked by stimulation of the right labyrinth were recorded from the ventrolateral funiculus on the right side at the first cervical segment of the spinal cord[14]. These discharges were effectively inhibited by conditioning stimulation with the cerebellar electrodes placed at a deep lamella of lobule IV or V (Fig. 1*a*), as plotted in Fig. 1*b* as a function of the conditioning–testing time intervals. As demonstrated previously[14], this inhibition represents the synaptic action of P cells on LVST cells. After destruction of the CDAO, the intensity of the P-cell inhibition of LVST cells gradually became much smaller (Fig. 1*b*). The time course of development of the depression is plotted in Fig. 1*c* for four experiments. In two control experiments, where lesions were placed in the rostral portion of the IO, leaving the CDAO intact, there was no depression in P-cell action such as seen in Fig. 1*c*(not shown).

In two experiments, IO destruction was replaced by local application of tetrodotoxin (TTX), which blocks impulse conduction but not axonal flows[15]. While 1 µl of saline solution containing 1.4 µM TTX was injected into the vicinity of the IO every hour or so, complete blockade of impulse activities in the IO was ensured by observing antidromic invasion of IO cells and/or trans-synaptic activation of P cells by stimulation of the IO. In both experiments, as demonstrated in Fig. 1*d*, TTX induced a rapid decline in the cerebellar inhibition but did not, however, reduce it to the extent seen after electrolytic IO destruction; instead, there was a partial recovery at 5–7 h after onset of TTX injection. This initial decline of the cerebellar inhibition was not reproduced by control injections of TTX-free saline solution, as shown by the broken line in Fig. 1*d*, and therefore apparently represents an effect specific to TTX.

Destruction of the IO also affected activation of P cells by CFA impulses. In Fig. 2*a*, the prominent negativity recorded from the molecular layer of the vermal cortex of lobule V represents trans-synaptic activation of P cells by CFAs stimulated in their intracerebellar course[1]. When the CDAO was destroyed together with the caudal portion of the medial accessory olive which projects to the medial part of the vermis[13], the activation of P cells gradually became much less effective in a

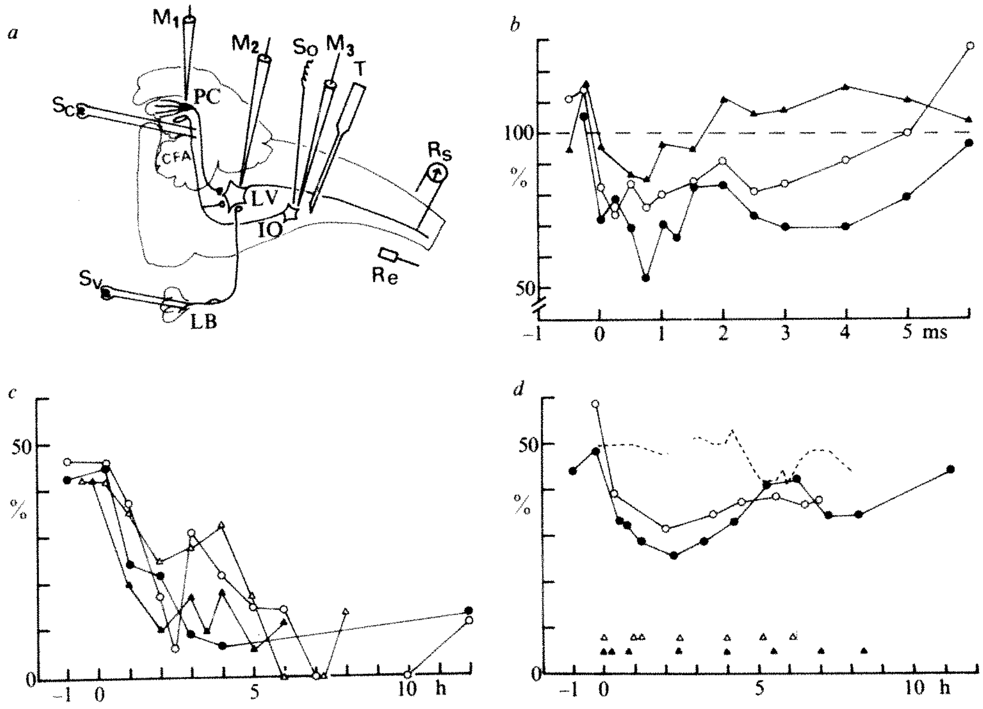

Fig. 1 Effects of destruction of, and local application of TTX to, the IO on P-cell inhibition of LVST cells. *a* illustrates schematically the experimental arrangements on a parasagittal section of the cerebellum and brainstem. PC, P cell; LV, LVST cell; LB, labyrinth; S_v, S_c, S_O and R_s, platinum–irridium needle electrodes, 0.2 mm in diameter, lacquered except for the very tips. One needle of R_s was inserted into the spinal cord, the other was placed on neck muscles. S_O was used for electrolytic destruction of IO. M_1, M_2 and M_3, glass microelectrodes filled with 2 M NaCl solution and having electric resistance of 1.5–2.5 MΩ. M_1 and M_2 were for recording from the cerebellar cortex and the nucleus of Deiters, respectively. M_3 was for examining antidromic activity of IO neurones under the influence of TTX; T, microsyringe containing 1.4 µM TTX-saline solution; R_e, indifferent electrode. *b* Plots amplitudes of LVST-cell discharges evoked by testing stimulation with S_v and recorded with R_s (ordinate), relative to their control size, as a function of the time interval between the conditioning stimulation with S_c and the testing one (abscissa). Three sets of plotted points were obtained before (●), and 4(○) and 12(▲) h after IO destruction in the same experiment. *c* Plots the reduction in LVST-cell discharges at the peak of the inhibition curves as in *b*, relative to their control size, as a function of time. IO destruction ended at 0 h. Results from four animals are superimposed. *d* Is similar to *c*, except for TTX application. For the two cases plotted by ○ and ●, times of TTX injection are indicated by △ and ▲, respectively. The broken line is for the control case where normal saline solution was injected every hour starting at 0 h.

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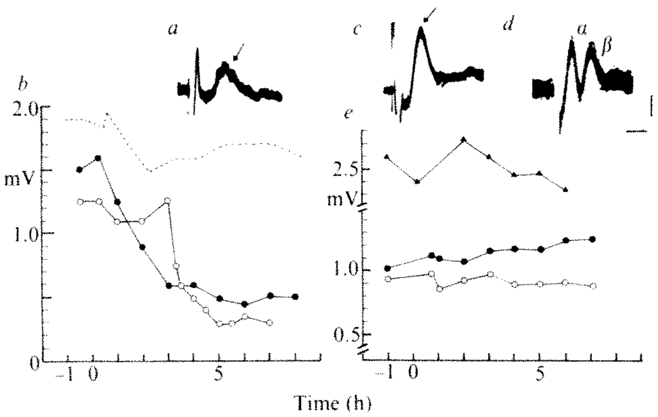

Fig. 2 Effect of IO destruction on CFA activation of P cells and electrical excitability of P-cell axons. *a*, Field potentials evoked by applying pulses of 0.1-ms duration and 0.5-mA intensity through S_c and recorded with M_1 (see Fig. 1*a*). Arrow marks the negativity representing activation of P cells by CFAs. *b*, Peak amplitudes for two animals plotted as a function of time before and after destruction of IO which ended at 0 h. The broken line indicates CFA activation of P cells in the presence of TTX, as shown in Fig. 1*d* (●, ▲). *c*, Field potentials evoked in the Purkinje cell layer by stimulation similar to *a* and recorded with M_1 optimally adjusted for recording antidromic activity of P cells (marked by an arrow). *d*, Field potentials evoked in the nucleus of Deiters by stimulation similar to *a* and *c*. α and β denote two spiky peaks. *e*, Plots amplitudes of antidromic activity of P cells (-▲-) and of α(●) and β(○) spikes, obtained from two animals as a function of time as in *b*. Records *a*, *c* and *d* were obtained by superimposing five successive sweeps repeated at a rate of 5 s^{-1}. For the measurement of these potentials for plotting in *b* and *e*, an average of over 20 successive sweeps was routinely done by use of a data processing computer (ATAC 501-10). Time scale indicated in *d* is 2 ms for *a* and 0.4 ms for *c* and *d*. Voltage scale is 1 mV for *a* and 0.4 mV for *c* and *d*.

time course comparable to that for depression of the cerebellar inhibition of LVST-cells, plotted in Fig. 2*b* for two cases. Local application of TTX induced only a small, somewhat transient depression in the activation of P cells, as indicated by a broken line in Fig. 2*b*.

By contrast, the excitability of P-cell axons was not affected by IO destruction. As shown in Fig. 2*c*, cerebellar stimulation by electrode S_c evoked a prominent negativity in the P-cell layer of lobule V which represented antidromic invasion of P cells. Field potentials similarly evoked but recorded from the nucleus of Deiters contained two spiky components of fast negativity (Fig. 2*d*), the earlier α spike representing volleys evoked in cerebellar afferent fibres and the later β spike those in P-cell axons[1]. Except for relatively small changes presumably arising from inadvertent movement of the recording electrode relative to brain tissues, none of these potentials exhibited systematic change following destruction of the IO (Fig. 2*e*).

The present investigation demonstrates that destruction of IO neurones causes a rapid failure in the excitatory transmission from CFAs to P cells and in the inhibitory transmission from the latter to LVST cells. These effects are specific to portions of the IO related to the vermal P cells and LVST cells, thus excluding the possible contribution of a nonspecific effect of damage to the medulla. As blockade of impulse activities of the IO with TTX produced only a relatively small, transient effect (Fig. 1*d*), the major effect of the IO destruction must be mediated by a non-impulse process, presumably an axonal flow in the CFAs. As impulse conduction in P-cell axons was not affected by IO destruction, it seems also that the postulated disturbance of axonal flow in CFAs affects the axonal flow in P-cell axons across CFA–P-cell synapses, eventually resulting in reduction of either release of the neurotransmitter, presumably GABA[16], from P-cell axon terminals, or postsynaptic reactivity to the neurotransmitter. It may be that P-cell synapses on LVST cells are affected through collateral of CFAs directly innervating LVST cells in the manner of the heterosynaptic interaction (see Fig. 1*a*).

Fast components of axonal flow have a velocity as high as 400 mm d^{-1} (ref. 17), and may travel from the IO to LVST cells through P cells in about an hour. This is fast enough to account for the rapid transfer of the effect of IO destruction to LVST cells, except for the short-term effect revealed within the initial hour (Fig. 1*c*). The short-term effect could be due to removal of impulses from CFAs and/or tissues surrounding the IO, as obtained by TTX blockade; P-cell inhibition of LVST cells might be influenced rapidly through a process such as impulse–axonal-flow interaction[18] in CFAs and/or P-cell axons, or through alteration of background activities of LVST cells, such as removal of the inhibition arising from the medullary reticular formation[19].

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Transducer noise in a photoreceptor

IN our attempts to unravel the workings of visual systems, we seek neural responses and interactions which can account for visual behaviour. A popular measure of visual performance is the threshold intensity at which a stimulus is detected with a given probability. To account for these detection tasks, one must not only measure neural signals at threshold[1,2], but also find the limiting sources of variance (that is, the noise) both within[3] and outside[4,5] the nervous system[6]. The manner in which the random nature of photon absorptions can limit the performance of visual systems is well understood[4,5,7,8]. When bleaching effects are negligible, photon absorptions follow the Poisson distribution. Consequently photon counts derived from the same mean signal have a variance equal to their mean. These fluctuations in counts are an inherent property of photon signals and are referred to as photon shot noise. Processes within the visual system generate intrinsic noise[7], but to assess its effect on thresholds one must first account for photon shot noise. This is generally difficult because photon catch cannot be measured directly, and must be estimated from optical parameters which are subject to significant error[7]. We have estimated the levels of photon shot noise and intrinsic transducer noise[9] in locust photoreceptors at low light intensities. We have chosen to search for sources of intrinsic noise in the visual system of the locust because it has recently been shown that, at low intensities, each effective photon produces a single, large quantum bump[10] (Fig. 1). Thus, we measure precisely the number of photons contributing to photoreceptor signals and derive, from the total response variance, that additional variance due to intrinsic noise. We report here that the transducer noise mimics the properties of photon

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Table 1 Analysis of photoreceptor response variance

Cell no.	Total response variance $(\bar{N}_c)^2$	Mean effective photons per flash $(\bar{N}_c)$ = predicted variance due to photon shot noise $(\bar{N}_c)^2$	Excess response variance due to transducer noise $(\bar{N}_c)^2$	Excess response variance due to transducer noise % Total	Mean response amplitude (mV)
1	10.0	5.8	4.2	42.0	6.4
	14.8	8.9	5.9	40.0	7.4
	24.1	14.1	10.0	41.4	9.2
	32.3	21.4	10.9	33.7	10.0
	69.0	31.6	37.4	54.2	11.7
	448.5	174.9	273.6	61.0	16.1
2	5.3	3.3	2.0	38.0	10.5
3	7.2	3.3	3.9	54.0	12.0
	27.9	9.2	18.7	67.0	17.0
	41.0	16.4	24.6	60.0	18.9
4	7.1	4.7	2.4	33.5	5.0
	27.6	13.5	14.1	51.0	9.0
5	25.9	11.4	14.5	56.0	6.4
6	21.4	10.5	10.9	51.0	10.0
7	65.3	27.8	37.5	57.4	11.9
8	25.3	14.8	10.5	41.4	7.0
	48.5	24.7	23.8	49.1	7.9
	88.5	37.7	50.8	57.4	10.9
				Mean = 49.3 ± 9.9%	

shot noise, is of similar magnitude, and, in most conditions, must have an equally significant role in determining thresholds.

To examine photoreceptor noise we use techniques perfected in previous experiments[9–12] and estimate receptor performance from intracellular responses to brief monochromatic light flashes (4 ms at 443 nm). Each stimulus falls within the linear integration time of the photoreceptor (which is >10 ms in a dark-adapted locust) so that response amplitude depends on the total number of effective photons delivered. Thus, the photoreceptor is used as a photon counter and problems associated with the statistical analysis of rate-dependent processes are avoided.

For each photoreceptor analysed we first count the number of bumps produced during prolonged periods of dim illumination.

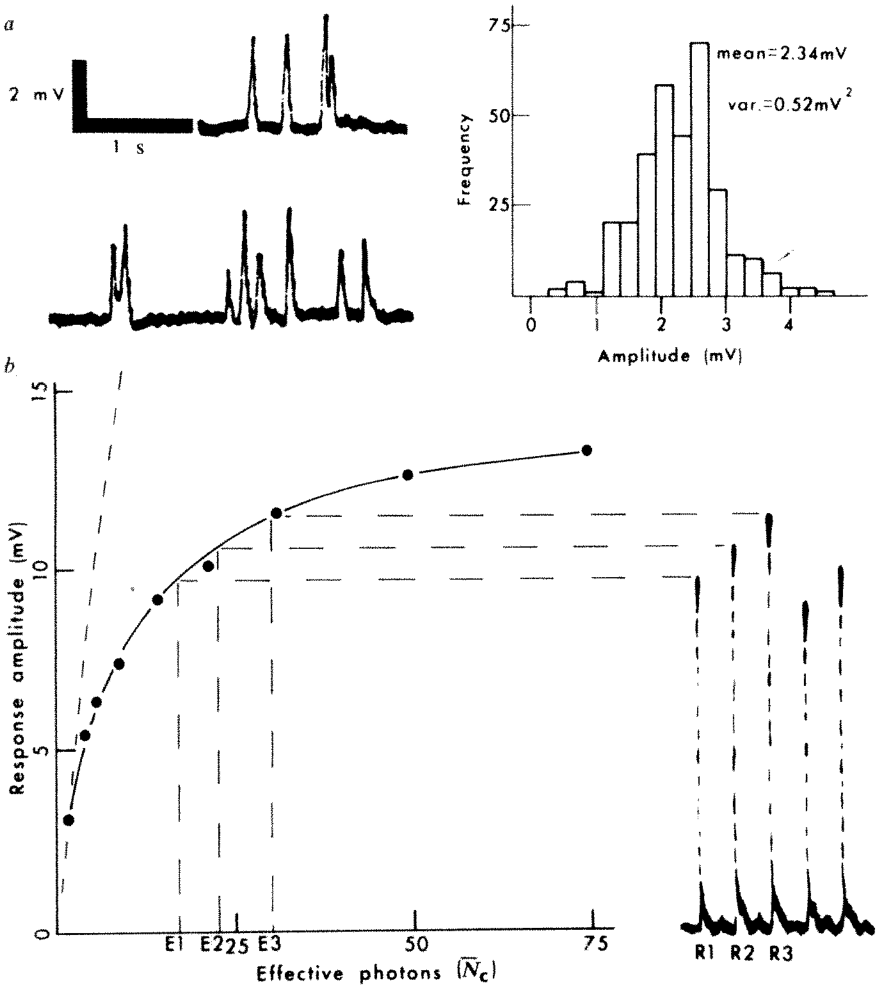

Fig. 1 Measurement of transducer noise in locust photoreceptors. *a* Shows quantum bumps recorded during dim illumination and their amplitude distribution. Their frequency is used to calibrate the effective quantal content of the flashes used to estimate response variance. *b* Demonstrates how an equivalent number of captured photons (E1, E2, E3, and so on) is attributed to responses (R1, R2, R3, and so on) induced by brief, higher-intensity flashes by referring all amplitudes to the intensity/response function. The intensity/response function predicted by the simple self-shunting model (- - -) is much steeper than the empirical (•—•).

This enables us to calculate the mean number of effective photons, $\bar{N}_c$, contained in higher-intensity flashes. Next, we derive the intensity/response function relating mean response amplitude, $\bar{V}$, to $\bar{N}_c$ (Fig. 1). Finally, we measure the amplitudes of response to 100–200 repeated flashes of known mean effective quantal content. Photon and intrinsic noise sources cause these responses to fluctuate in amplitude (Fig. 1) and response variance is measured in units of equivalent effective photons (EEPs) squared by converting every voltage amplitude to an equivalent mean photon count[9,12] using the intensity/response function (Fig. 1). The response variance due to photon shot noise equals $\bar{N}_c$ (from Poisson statistics). The additional response variance due to intrinsic noise is derived by subtracting the photon shot noise from the total response variance. Thus, the degrading effects of intrinsic noise are measured in terms of incident intensity.

Our results (Table 1) show that over a range of 1.7 log units intensity, the random absorption of photons only accounts for 33–67% of total response variance. The remaining fluctuations must be generated within the photoreceptor itself. At low intensities this intrinsic noise is multiplicative, producing variance proportional to mean photon catch, $\bar{N}_c$, in the range $\bar{N}_c = 6$–30. At higher intensities our preliminary observations suggest that intrinsic noise increases in magnitude relative to photon shot noise (Table 1).

We are confident that this intrinsic noise is not an artefact generated by errors in experiment or calibration. Full details of the calibration of the Uniblitz high speed shutter, the constant current tungsten light source, and the interference and attenuating filters are given elsewhere[10]. Calibrations gave consistent results which accurately predicted both relative quantum bump frequencies at different steady intensities and the fraction of successful responses produced by different threshold flash intensities. The accuracy of our method depends on the determination of relative, rather than absolute, stimulus energies, because each cell's quantum catch is measured from bump counts. Rigorous controls ensured that we rejected data from cells whose sensitivity changed during an experiment. A determination of quantum capture efficiency and the intensity/response function preceded and followed every estimation of response noise, which was measured several times at each intensity. The inter-stimulus interval was adjusted to prevent light adaptation. During a single experiment, the resting potential drifted by <0.5 mV (~1%).

We can dismiss several sources of intrinsic noise in the dark-adapted locust photoreceptor. In total darkness, 'spontaneous' photon signals occur at a rate of less than 1 every 360 s (ref. 10) (this is 1 every 160 s for dogfish rods[13]) and will contribute negligible variance to responses to brief flashes. Membrane voltage noise in the dark (400 μV peak-to-peak) is also negligible relative to bump amplitude and this contrasts strongly with the barrage of dark noise in rods and cones[14]. Consequently, in locust photoreceptors, the intrinsic noise described above must be associated with the light response, and so is termed transducer noise[9]. One of us (P.G.L.) has recently eliminated three potential sources of this transducer noise: there is no variance associated with the number of bumps produced by an effective photon[10]; bump size seems to be independent of photon wavelength[11], even in cells containing two or more photopigments, implying that each photopigment produces equal-sized bumps; there is no variance associated with site of photon capture (geometric noise).

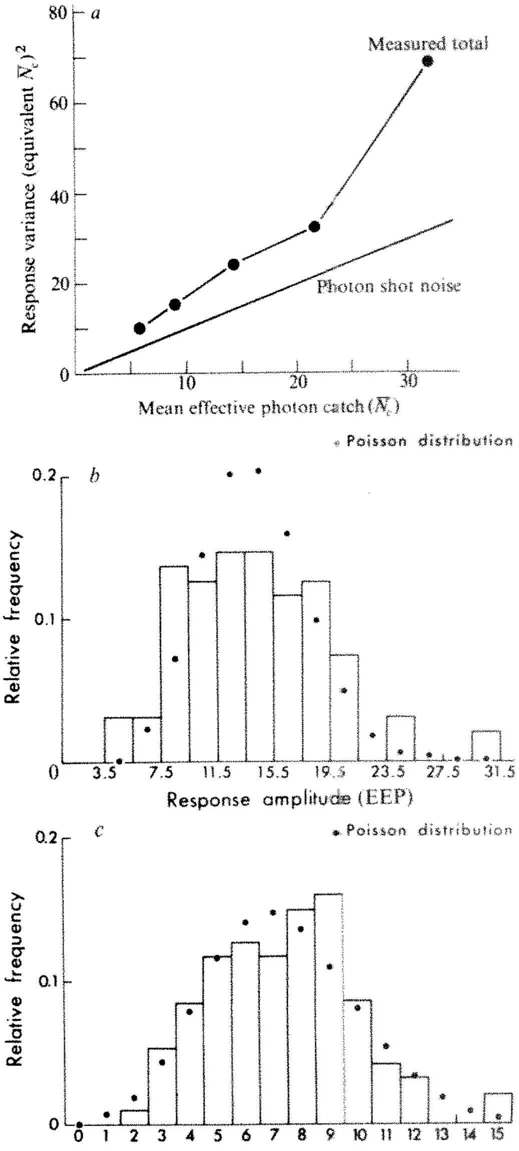

Fig. 2 *a*, Total response variance measured in squared equivalent effective photons (EEPs) plotted against mean effective photon catch per flash. For small responses the total variance is proportional to mean intensity and in this sense mimics photon shot noise. *b*, The frequency distribution of response amplitudes to one series of constant-intensity flashes. Response amplitudes are expressed in EEPs using the methods described in the text. The distribution of amplitudes is broader than that predicted by Poisson statistics from the mean effective photon catch estimated from bump counts (●). This extra scatter is due to transducer noise. *c*, An amplitude frequency distribution plotted from the same data as used in *b*, but here the shot noise analysis described in previous studies[9,12] has been used to assign to each response an effective quantal content. This method assumes that all variance is generated by photon shot noise and calculates the mean quantal content of stimuli from the signal-to-noise ratio of response amplitudes[9] (expressed in equivalent intensity). Again, response noise mimics photon shot noise because the distribution of apparent photon catches is Poissonian. However, intrinsic noise has decreased the signal-to-noise ratio and lowered the apparent photon catch. All data are taken from cell 1, Table 1.

Two known manifestations of transducer noise are the fluctuations in amplitude (Fig. 1) and latency of single photon responses[10]. With respect to amplitude fluctuations, the simplest assumption is that apart from overall response compression, bumps add independently to each other. On this basis one would expect the slope, S, of variance against mean (in terms of EEPs) to be: $S = 1 + (\text{variance(bump amplitude)}/(\text{mean(bump amplitude)})^2)$ (see ref. 15), which is always less than observed; for example, the predicted slope for cell 1, Table 1 is 1.1 rather than the observed value of 1.5–2.0. The disparity presumably indicates that such a simple assumption is not justified. This is suggested by the observation that locust photoreceptors do not possess 'simple' self-shunting membranes (for review see ref. 16) for which the above equation for slope, S, was derived[15]. In this case, total conductance change is proportional to photon catch and a hyperbolic intensity/response function is predicted which greatly overshoots the empirical one (Fig. 1). Thus, a better understanding of the mechanisms which produce this severe response compression seems necessary to assess the effects of bump amplitude variability. Initial modelling of the effects of variable bump latency using Monte Carlo methods suggests that it causes very little response compression and contributes about 10% to observed transducer noise. Clearly, we require a better knowledge of transduction if we are to determine the extent to which the unequal responses to single photon absorptions seen from bumps, contributes to transducer noise.

Can transducer noise influence neural and behavioural thresholds? At intensities so low that bumps do not fuse, single photon hits could be registered in a manner which avoids transducer noise. For example, given the high voltage gain found at the first synapse in the compound eye (reviewed in ref. 17), the second-order cells might produce brief saturating responses for each photoreceptor bump received. However, at higher intensities, bumps fuse and a receptor can no longer act as a perfect photon counter in this manner. Transducer noise blurs the discrete jumps in amplitude resulting from additional photon absorptions, and contributes noise indistinguishable in the intensity domain from photon shot noise (Fig. 2*b*, *c*). Arguments which suggest that the proportionality of increment thresholds to (intensity)$^{1/2}$ indicate a photon-counting system unaffected by intrinsic noise[8], are here demonstrated to be false, because we describe a source of intrinsic noise (transducer noise) which is multiplicative. Thus, although photoreceptor noise has a large intrinsic component, it mimics the statistical properties expected if photon shot noise alone was present[6]. Our measurements suggest that in most conditions transducer noise must be as significant as photon shot noise in determining visual thresholds. Consequently, transducer noise must be considered with other sources of intrinsic noise when accounting for visual performance, for the design of photoreceptors, and for higher-order strategies for optimum sampling of the visual signal[18].

During the revision of this letter, a report was published which suggested that multiplicative noise might degrade human visual performance without distorting the underlying Poisson statistics of photon capture. This would account for the number of quanta apparently detected falling far short of estimated capture by the photopigment[19]. Our conclusions about the effects of transducer noise are clearly similar and establish a precedent for the existence of multiplicative noise within a visual system.

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Association of cellular membrane of *E. coli* minicells with the origins/terminus region of replication of plasmid ColEl DNA

COLICINOGENIC plasmid El (ColEl) can be isolated from *Escherichia coli* as a supercoiled DNA molecule of 2.14 μm long or as a supercoiled DNA–protein complex[1]. Certain protein denaturing agents will convert this complex to an open circular (relaxed) DNA form with one single-strand break in the heavy (polyUG binding) strand of the duplex ColEl DNA (refs 2, 3). The induced single-strand break in ColEl DNA is located approximately 19% of the length of ColEl DNA from the single *Eco*RI restriction endonuclease site in the plasmid[4]. Analysis of ColEl replicating intermediates has shown that replication proceeds unidirectionally from an origin located near the position of the relaxation complex nick, approximately 19% from the single *Eco*RI restriction site[5–7]. The site of the relaxation-complex nick has been located approximately 300 nucleotides away from the origin of ColEl DNA replication[9]. The nucleotide sequence of the origin region of ColEl DNA[8,9] and of a small ColEl-type plasmid[10] have been determined. A model for the possible role of the relaxation complex in ColEl DNA replication and/or conjugal transfer has been presented[11]. A major feature of this model is that the relaxation complex proteins may help promote association of plasmid DNA with a replicator and/or conjugal transfer site on the bacterial membrane. We describe here an electron microscopic analysis of *Eco*RI-cleaved ColEl and mini-ColEl DNA that were purified as stable DNA–membrane complexes from *E. coli* minicells. Evidence is presented that membrane-like structures attach to ColEl DNA and mini-ColEl DNA in a region that includes both the site of the origin/terminus of DNA replication and the site of single-strand cleavage by the relaxation complex.

Minicells that contain plasmid ColEl DNA or a hybrid mini-ColEl plasmid containing a kanamycin (Km) resistance fragment (pML21)[13] were purified from *E. coli* P678-54, Thy$^-$. The plasmid DNA in the purified minicells was labelled with ^{3}H-thymidine, the minicells were lysed, and the lysate centrifuged on caesium chloride–sucrose gradients[12]. Figure 1*a* shows that in the conditions of centrifugation the ^{3}H-ColEl DNA from minicells separates into two major peaks, one which rapidly sediments to an isopycnic density of about 1.35 g ml^{-1} (pool *A*), the other remaining near the top of the gradient. A visible band of opalescent material, presumably cellular membrane fragments, is present in the position of pool *A*. To show that pool *A* contains membrane components, cultures of *E. coli* P678-54 harbouring ColEl were labelled with ^{3}H-leucine or [2-^{3}H]glycerol, which are incorporated into protein and membrane phospholipid, respectively[14]. Minicells were purified from these cultures and treated exactly as before, except the cells were not labelled with ^{3}H-thymidine. It was found (data not shown) that 17% of the total ^{3}H-leucine in the lysed minicells is located in the 1.35 g ml^{-1} density region in the caesium chloride–sucrose gradient. In addition, 100% of the phospholipid label reached equilibrium at a density of 1.35 g ml^{-1}. Therefore, the observed isopycnic density of the ^{3}H-ColEl DNA is most probably accounted for by the association of the plasmid DNA with membranous components.

Purified supercoiled, open circular, or linear ^{14}C-ColEl DNA was added to the lysate at various stages of the lysis procedure. Figure 1*a* shows that supercoiled ^{14}C-ColEl DNA does not appear in the pool *A* region of the gradient, suggesting that little or no ^{3}H-ColEl DNA from the minicell lysate aggregated nonspecifically to components in this peak. Similar results occurred with purified circular and linear ^{14}C-ColEl DNA. As shown in Fig. 1*b*, when pool *A* was dialysed and centrifuged a second time on a caesium chloride–sucrose gradients, approximately 50% of ^{3}H-ColEl DNA in pool *A* remained near the top

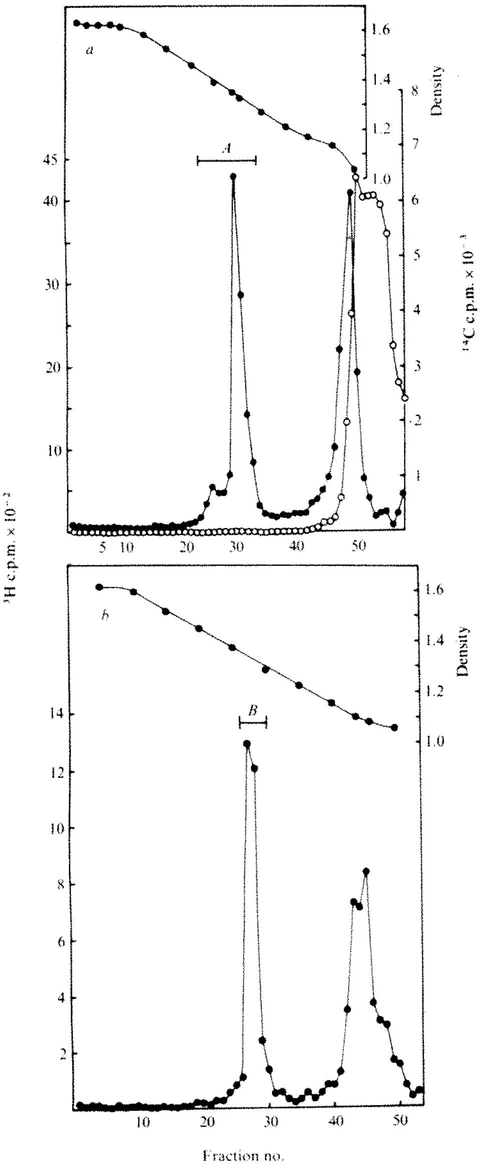

Fig. 1 Caesium chloride–sucrose gradients of lysates of minicells containing ^{3}H-labelled plasmid ColEl DNA. *a*, *E. coli* minicell producing strain, P678-54, Thy^{-}, carrying plasmid ColEl was grown to late exponential phase in 3 l of LB medium. The culture was centrifuged at 1,500 r.p.m. for 3 min in a Sorvall GSA rotor and the supernatant pelleted at 8,000 r.p.m. for 10 min, followed by resuspension in BSG (0.85% NaCl, 0.03% KH_2PO_4, 0.06% Na_2HPO_4, 0.1% gelatin). The resuspended minicells were layered on 33 ml 5–20% sucrose gradients and centrifuged in an HB4 rotor at 4,500 r.p.m. for 15 min. The minicell band was removed, diluted with BSG, pelleted, and again centrifuged on 5–20% sucrose gradients. This was repeated 2 or 3 times until *E. coli* whole cells could no longer be seen in the minicell preparation by light microscopy. ColEl DNA in the purified minicells was labelled with ^{3}H-thymidine by incubation for 30 min at 37 °C in 25 ml of M9 medium containing 0.1% vitamin-free casamino acids, 0.2% glucose, 20 μg ml^{-1} thiamine, 50 μg ml^{-1} tryptophan, 0.002 M cyclic AMP and 2 μg ml^{-1} each of adenosine, deoxyadenosine, guanosine, deoxyguanosine, cytosine, deoxycytosine and uridine, and 1 mCi of ^{3}H-thymidine. Incorporation of ^{3}H-thymidine into trichloroacetic acid-insoluble material was linear up to 90 min. After labelling, the minicells were centrifuged and resuspended in 2 ml of 0.75 M sucrose. Purified ^{14}C-thymine-labelled ColEl supercoiled DNA was added immediately after sucrose as a control for nonspecific association of ColEl DNA with membrane components. The mixture was incubated for 15 min on ice with 100 μg ml^{-1} lysozyme and 0.01 M EDTA, after which Triton X-100 was added to 0.05%. The lysate was layered on a preformed linear gradient of 5 M caesium chloride plus 20% sucrose to 0.5 M caesium chloride plus 10% sucrose made in TES (0.1 M Tris, *p*H 8.0, EDTA, *p*H 8.0, 0.5 M NaCl, 0.005 M) and centrifuged in an SW 27 rotor at 22,000 r.p.m. at 4 °C for 30 min[12]. Fractions were collected from the bottom, portions counted, and the refractive index of each fraction determined. The density was determined from caesium chloride–sucrose standard curves of density against refractive index. ●, ^{3}H radioactivity; ○, ^{14}C radioactivity. *b*, Pool A from the caesium chloride–sucrose in *a* was dialysed against TES and centrifuged on a caesium chloride–sucrose gradient in the same conditions as above.

of the gradient. Dialysis and centrifugation of pool *B* (Fig. 1*b*), again in the same conditions, does not result in any loss of ColEl DNA from the 1.35 g ml^{-1} density region, indicating that the remaining 20–25% of the total radioactivity incorporated into ColEl DNA (pool *B*) is stably associated with membrane material. In different experiments the percentage of total ^{3}H-thymidine incorporated into ColEl DNA found in pool *A* (Fig. 1*a*) varied, but the percentage of incorporated ^{3}H-thymidine remaining in pool *B* (Fig. 1*b*) was usually 20–25%. The released ^{3}H-ColEl DNA near the top of the gradient may represent plasmid DNA either specifically but weakly associated with membrane components or nonspecifically-associated DNA that is released during handling.

As a further test of the stability of ^{3}H-ColEl DNA membrane association, pool *B* was subjected to various treatments. It was found that ColEl DNA can be released from membrane material, as determined by a change in density of the ^{3}H-DNA in caesium chloride–sucrose gradients, by treatment with pancreatic DNase or the ionic detergents Sarkosyl and SDS (0.2%). Treatments that did not release the plasmid DNA included exposure of 2M NaCl, Triton X-100 (0.2%), pancreatic RNase (1 mg ml^{-1}), phospholipase A, freezing to −20 °C, and heating to 60 °C for 10 min. Treatment with pronase or heating at 100 °C for 10 min. gave variable levels of dissociation of the complex.

ColEl DNA–membrane complexes from pool *B* (Fig. 1*b*) and mini-ColEl-Km (pML21) DNA–membrane complexes from similar preparations were examined by electron microscopy.

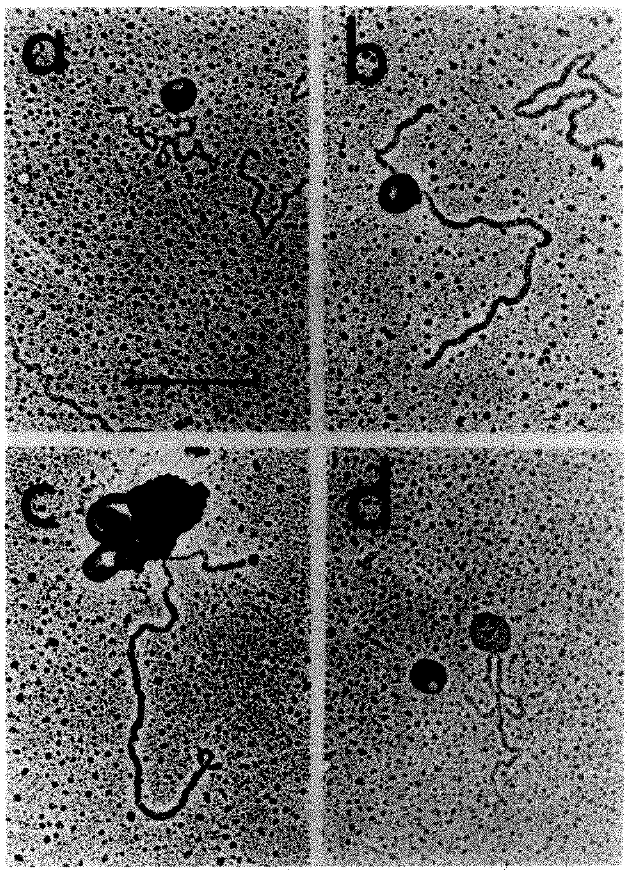

Fig. 2 Electron micrographs of *Eco*RI-cleaved ColEl DNA–membrane complexes and mini-ColEl DNA–membrane complexes. Minicells containing ColEl DNA or mini-ColEl-Km DNA were purified, labelled, lysed and centrifuged on caesium chloride–sucrose gradients as described in Fig. 1. Fractions corresponding to Pool *B* in Fig. 1 were dialysed against TES. Aliquots were adjusted to 10 mM $MgCl_2$ and either incubated at 30 °C for 30 min as controls for nuclease contamination or incubated at 30 °C for 30 min with *Eco*RI. Control and cleaved samples were spread for electron microscopy as previously described[16]. Purified open circular ColEl DNA was spread with the samples as a size reference. a, Supercoiled ColEl DNA–membrane complex; b, c, *Eco*RI-cleaved ColEl DNA–membrane complex; d, *Eco*RI-cleaved mini-ColEl DNA–membrane complex. The bar in frame a is equal to 0.5 μm.

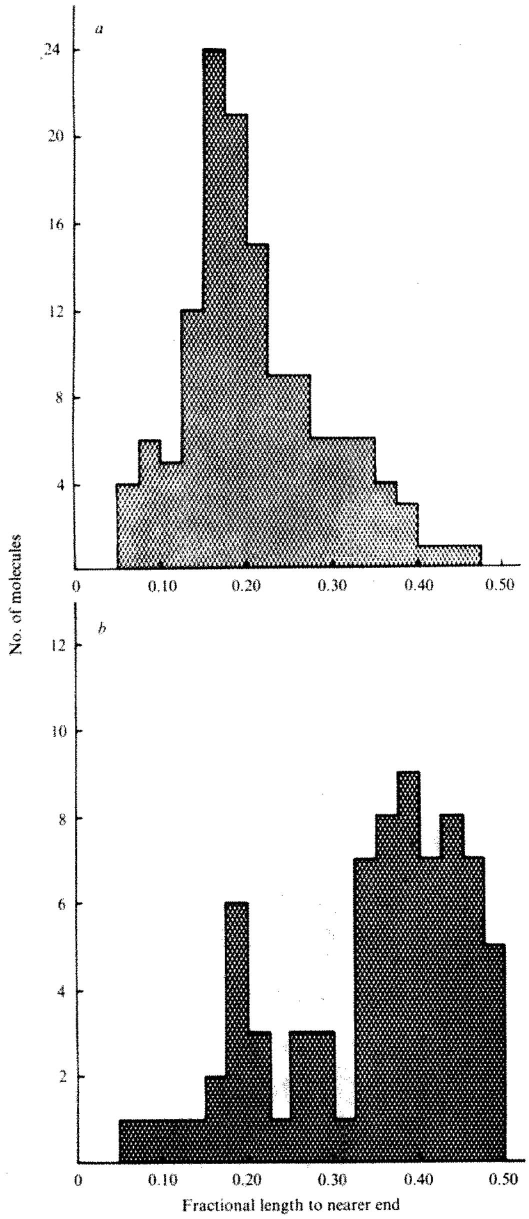

Fig. 3 Histogram of locations on *Eco*RI-cleaved ColEl DNA and mini-ColEl DNA in contact with a membrane fragment. Samples of purified ColEl DNA or mini-ColEl–Km DNA analogous to pool *B* of Fig. 1 were cleaved with *Eco*RI and visualised by electron microscopy. Molecules were measured in relation to purified circular ColEl DNA spread on the same grid, and only molecules that were of unit length were considered for analysis. Measurements were made on the short arm of the restricted DNA from the *Eco*RI site to the membrane fragment. Molecules with large membrane fragments covering the DNA in more than one of the arbitrary divisions of 0.025 molecular length were included in the histogram in each division in contact with membrane material. *a*, ColEl DNA; *b* mini-ColEl–Km DNA.

Figure 2 shows complexes before and after cleavage by *Eco*RI restriction endonuclease. This analysis usually showed some membrane-like fragments free from DNA in addition to supercoiled, open circular, and, in the case of restricted samples, linear plasmid DNA membrane complexes. Over 50% of the linear ColEl DNA observed was associated with a membrane fragment. A low percentage (<5%) of the membrane-bound ColEl DNA had two fragments attached. Most DNA-bound membrane fragments appeared vesicular and seemed to be of two general types (Fig. 2*a*, *b*, *d*). These fragments were about 0.1 μm in diameter. Other larger fragments could also be seen (Fig. 2*c*). When pool *B* was treated with 0.2% Sarkosyl and dialysed before preparation for electron microscopy, membrane-like structures could not be seen. As mini-ColEl-Km DNA has two *Eco*RI restriction sites, restricted preparations showed two molecules, the 4.5×10^6-dalton Km restriction fragment and the 2.2×10^6-mini-ColEl plasmid. Less than 3% of the Km DNA fragment possessed an attached membrane fragment. However, a large proportion of the mini-ColEl DNA was associated with a membrane fragment.

The total lengths of the linear molecules associated with membrane fragments derived from *Eco*RI-cleaved DNA of pool *B* were determined by electron microscopy. For ColEl DNA, only molecules that were the same length as open circular ColEl DNA spread on the same grid square were further analysed. For mini-ColEl only linear DNA molecules that were 1.2 μm in length when compared to the control ColEl molecules spread on the same grid square were further analysed. The location on the DNA molecule of the membrane fragments relative to the *Eco*RI restriction sites was measured. A total of 75 ColEl molecules from three different preparations and 50 mini-ColEl molecules from two different preparations were measured. Figure 3*a* and 3*b* represent, respectively, the number of ColEl and mini-ColEl molecules that had a membrane fragment in contact with the DNA within arbitrarily chosen divisions of 0.025 fractional length of the DNA molecule. If a membrane fragment was in contact with a DNA molecule in more than one of the divisions of 0.025 fractional length it was counted and plotted in each division. Some of the scatter and broadness of the histogram peaks may be due to nonspecific associations or to large membrane fragments that cover disproportionately large regions of the DNA molecules. As salt concentrations of ≥2 M will dissociate most DNA binding proteins[15], the caesium chloride–sucrose gradient conditions probably dissociate most weak associations.

Figure 3*a* indicates that about 60% of the ColEl DNA-bound membrane fragments are in contact with the DNA between 0.15–0.22 fractional length of the *Eco*RI restricted plasmid. This corresponds to the region of origin/terminus of replication and to the relaxation complex site of ColEl DNA. Figure 3*b* shows that about 55% of mini-ColEl DNA-bound membrane fragments are found at 0.33–0.45 fractional length of the *Eco*RI cleaved molecule. This is in the region of the origin of DNA replication of the mini-ColEl molecule (0.36–0.40 fractional length of *Eco*RI-cleaved mini-ColEl (R.B.S. and R. Eichenlaub, unpublished). Although this analysis does not distinguish between the two ends of the DNA molecules, the close correspondence to the origin of replication suggests a direct involvement of membrane components in plasmid ColEl DNA replication. The failure to isolate mini-ColEl DNA in the form of relaxation complex[13] may indicate in the case of this plasmid that the association with the membrane material is more a property of the origin/terminus of replication or the relaxation complex site of nicking than of the active relaxation complex proteins.

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A peptide resembling COOH-terminal tetrapeptide amide of gastrin from a new gastrointestinal endocrine cell type

THE gastrointestinal hormones gastrin and cholecystokinin (CCK) share identical COOH-terminal sequences. In both hormones the COOH-terminal tetrapeptide amide constitutes the biologically active region, and the rest of the two molecules determines their potency and selectivity for different target cells. Their similarity in structure and activity may reflect a common phylogenetical origin[1]. The COOH-terminal tetrapeptide amide is highly immunogenic in each hormone and many gastrin antisera are specific for this sequence and also bind CCK. In order to study the hormones by immunocytochemistry and radioimmunoanalysis, antisera specific for several distinct sequences must be used. Previously, we have used such antisera to characterise antral gastrin cells and intestinal cholecystokinin cells by immunocytochemistry[2]. We now report the identification of three distinct types of gastrointestinal cells which show COOH-terminal gastrin (or CCK) immunoreactivity. One type corresponds to gastrin-producing G cells, another to CCK-producing I cells and a third type to TG cells which react only with antisera against the COOH-terminal tetrapeptide.

Specimens from the gastrointestinal tract of monkeys ($n = 5$) and pigs ($n = 10$) were studied by immunocytochemistry and correlative electron microscopy. The antisera used included As. 1295, 2717 and 4562, raised in rabbits against synthetic human gastrin-17, As. 4710 raised in a rabbit against the synthetic mid-portion (6–13) of human gastrin-17, As. 4478 raised in a guinea pig and As. 4698 raised in a rabbit against porcine CCK-33. As previously documented[1,2] As. 1295 recognises the NH_2 terminus of human gastrin-17, As. 4710 the mid-portion of human gastrin-17, As. 4562 and 2717 bind the COOH-terminal tetrapeptide portion of both gastrin and CCK, As. 4698 binds the region (25–30) of triacontatriapeptide CCK (CCK-33) and so also recognises octapeptide CCK (CCK-8) and caerulein, and As. 4478 binds the region (19–25) of CCK-33 and does not recognise CCK-8. The region-specificity of the antibodies is

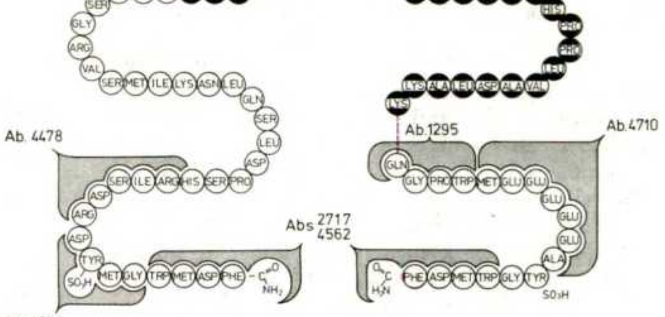
NH₂
TYR ILE GLN GLN ALA ARG LYS ALA PRO SER GLY ARG VAL SER MET ILE LYS ASN LEU GLN SER LEU ASP PRO SER HIS ARG ILE SER ASP ARG ASP TYR MET GLY TRP MET ASP PHE
SO₃H
NH₂
Ab. 4478
Ab. 4698
Abs 2717 4562
NH₂
R Comp. I
GLP LEU GLY LEU GLN GLY HIS PRO PRO LEU VAL ALA ASP LEU ALA LYS LYS GLN GLY PRO TRP MET GLU GLU GLU GLU GLU ALA TYR GLY TRP MET ASP PHE
H₂N
SO₃H
Ab. 1295
Ab. 4710

Fig. 1 The amino acid sequences of porcine CCK-33 and gastrin-34 and the region-specificity of the antisera used in this study.

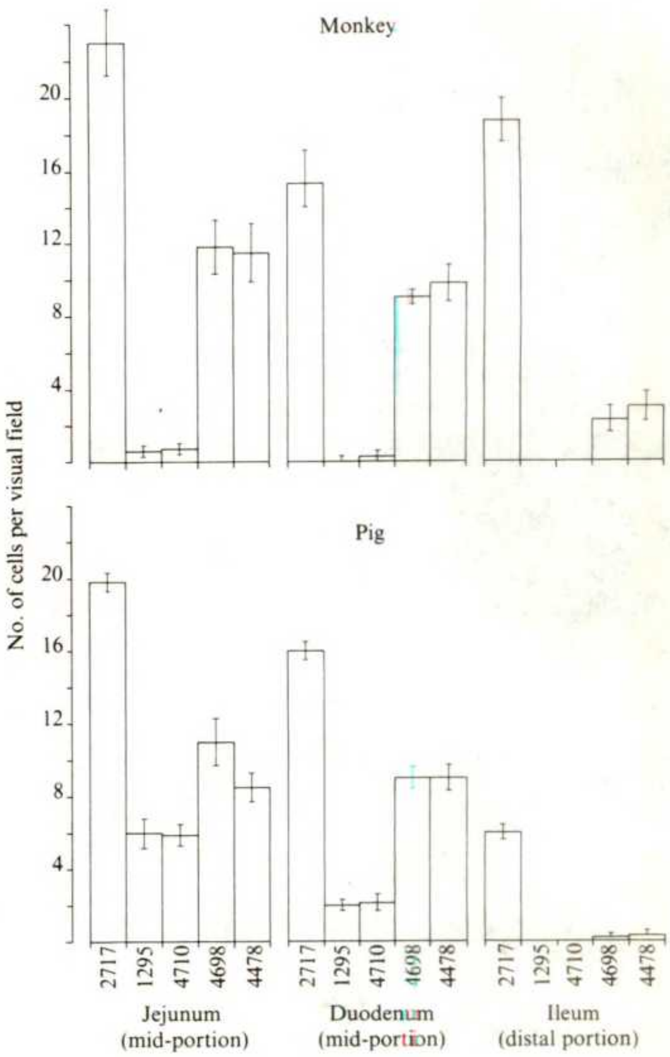

Fig. 2 Frequency distribution of intestinal endocrine cells reactive with COOH terminus-specific (Abs 2717 and 4562), CCK-specific (Abs 4478 and 4698) and gastrin-specific (Abs 1295 and 4710) antibodies. Note that in all parts of the small intestines the number of COOH terminus-reactive cells exceeds the number of gastrin and CCK cells. This difference reflects the number of TG cells. Pig ($n = 10$) material was collected at a nearby abattoir and green monkeys ($n = 5$) were anaesthesised with Mebumal (pentobarbital). Specimens from the gastrointestinal tract were frozen in melting Freon-22, freeze-dried, vapour-fixed in paraformaldehyde and embedded in paraffin. Serial 3-μm sections were reacted with antibodies detecting the COOH terminus (Abs 2717 and 4562), the 6–13 region (Ab. 4710) and the NH_2 terminus (Ab. 1295) of human gastrin-17 as well as antibodies detecting regions 19–25 (Ab. 4478) and 25–30 (Ab. 4698) of porcine CCK-33 (ref. 1). The antisera were immunoabsorbed and then stained as described previously[1,2]. Specificity was checked by conventional staining controls and by absorptions against synthetic human gastrin-17, the synthetic mid-portion (6–13) of human gastrin-17, pentagastrin, 99% pure porcine CCK-33, caerulein and other gut peptides[1,2]. The site of antigen–antibody reaction was revealed by the peroxidase–antiperoxidase (PAP) procedure of Sternberger or by immunofluorescence[7]. For quantitation, serial sections were stained with the different antisera (PAP technique only), so that the first and last slides in one series were stained with the same antiserum. The variation between these two slides never exceeded, and was usually considerably below, 5%. Only perfectly transverse sections were counted and the results were evaluated as numbers of cells per field of vision (objective × 10, eye-piece × 12.5) or as absolute figures (data not shown), The total surface area of the (mucosal) sections averaged 0.25–0.5 cm^2. The number of cells was always counted in corresponding adjacent visual fields.

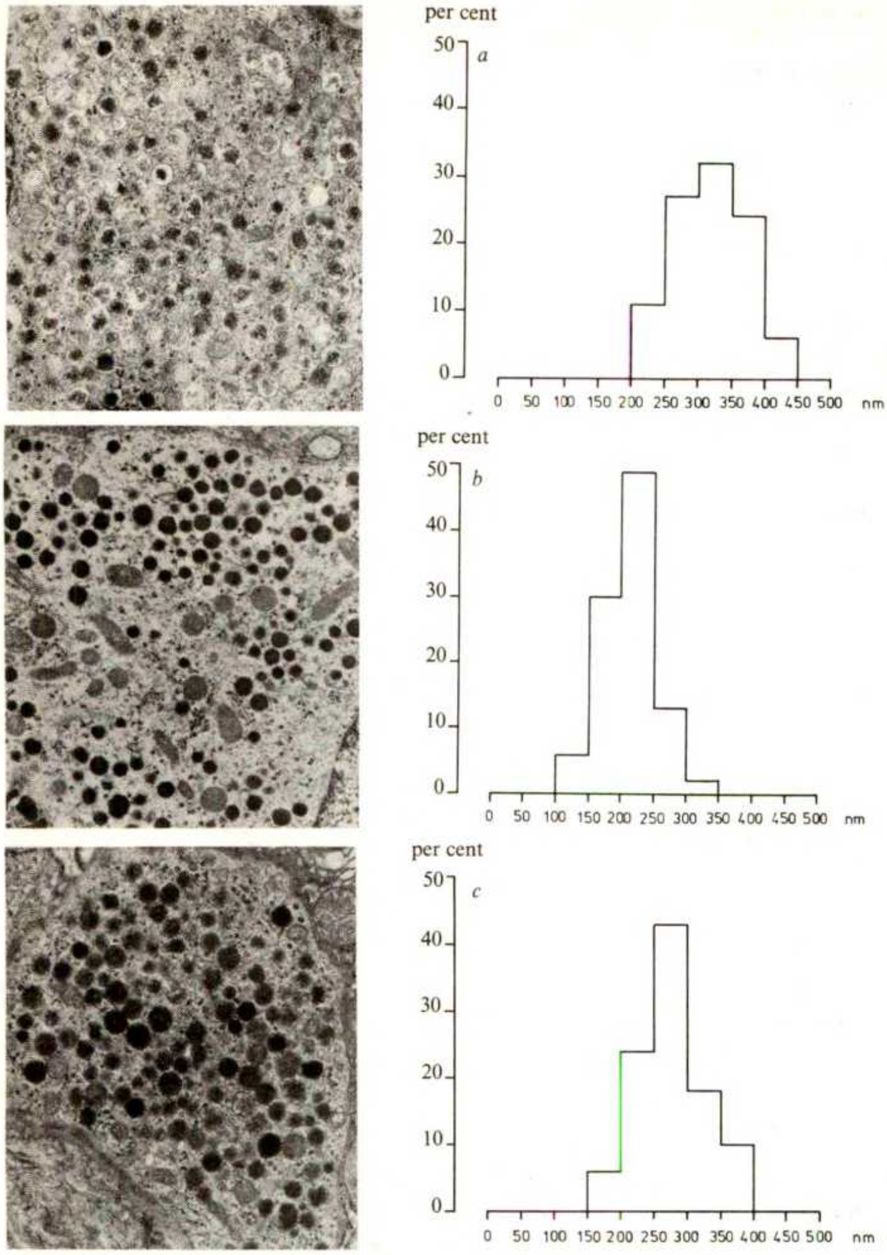
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Fig. 3 Electron micrographs and size distribution histograms of cytoplasmic granules in *a*, gastrin (G) cells; *b*, CCK (I) cells; and *c*, TG cells of green monkeys. Note the differences in size and morphology of the three granule types. (×7,100). Specimens from the antropyloric and duodenal mucosa of green monkeys ($n = 3$) were fixed by immersion for 15 min at 4 °C in a 2% glutaraldehyde: 3% formaldehyde solution in 0.1 M sodium phosphate buffer *p*H 7.3. After washing in buffer and osmication the specimens were embedded in Epon 812 and adjacent semithin and ultrathin sections were cut. Semithin sections were deplasticised and de-osmicated and permitted to react with the region-specific antisera[8] (see Fig. 1). The site of antigen–antibody reaction was revealed by immunofluorescence and immunoreactive cells were identified in the adjacent ultrathin sections. Controls were as described in Fig. 1.

shown schematically in Fig. 1. As. 1295, 4710, 4698 and 4478 contained additional populations of COOH terminus-reactive antibodies which were removed by immunoabsorption against either CCK-33 (As. 1295 and 4710) or synthetic human gastrin-17 (As. 4698 and 4478). Following the immunoabsorption step the sequence-specificity of the antibodies was verified by absorptions against synthetic human gastrin-17, the synthetic (6–13) region of human gastrin-17, pentagastrin (Peptavlon) synthetic caerulein and 99% pure porcine CCK-33 and by the inability of As. 4698 and 4478 to react with antral gastrin cells, by the inability of As. 1295 and 4710 to react with intestinal CCK cells and by the ability of As. 4562 and 2717 to demonstrate both gastrin and CCK cells, as reported elsewhere in detail[1,2]. Our immunocytochemical results were quantitied as described in Fig. 2.

Use of gastrin-specific antisera (As. 1295 and 4710) revealed that gastrin cells predominated in the antropyloric mucosa and were few in the small intestines (Fig. 2). The oxyntic mucosa of the stomach, the Brunner glands of the duodenum and the distal ileum only occasionally contained gastrin cells (Fig. 2). The colon was devoid of such cells.

The CCK-specific antisera (As. 4698 and 4478) demonstrated numerous endocrine cells in the duodenum and jejunum. The proximal ileum contained a few scattered CCK cells, whereas the stomach, the duodenal glands of Brunner and the distal ileum were devoid (monkeys) or nearly devoid (pigs) of such cells (Fig. 2).

The COOH terminus-specific antisera (As. 2717 and 4562) demonstrated both gastrin and CCK cells, as well as a third population of intestinal endocrine cells, which failed to react with the gastrin- or CCK-specific antisera. The existence of the third cell population was inferred from the finding that the COOH terminus-specific antisera demonstrated many cells in both the Brunner gland area and in the distal ileum. Cell counts (Fig. 2) verified the existence of the third cell type, the sum of COOH terminus-reactive cells greatly exceeding the sum of gastrin and CCK cells. Thus, COOH terminus-reactive cells, distinct from gastrin and CCK cells (TG cells), were few in the antropyloric mucosa and numerous in the small intestines. They were particularly well represented in the Brunner glands of the duodenum and in the distal ileum, areas in which gastrin and CCK cells were virtually absent (Fig. 2). TG cells were not detected in the oxyntic mucosa or in the colon.

Identification of gastrin, CCK and TG cells by electron microscopy was carried out in the monkey antropyloric and duodenal mucosa using the region-specific gastrin and CCK antibodies. Antral gastrin cells were characterised by the presence of numerous cytoplasmic granules of variable electron density. Most granules contained an irregular dense core and measured 221–442 nm (mean 307 nm, see Fig. 3). The cells reached the lumen through an apex endowed with microvilli, and contained organelles typical of peptide- or protein-synthesising cells. This ultrastructure is similar to that of G cells in other species[3,4].

Duodenal CCK cells were elongated or flask-shaped and contained numerous membrane-bound granules with a thin electron-lucent halo separating the highly electron-dense core from the membrane. The granules were round or slightly angular and measured 128–383 nm (mean 209 nm, see Fig. 3). Their ultrastructure is similar to that of the I cells of the small intestines, which have been shown to produce CCK[5].

The TG cells were identified in both the antropyloric gland area and in the duodenum. They contained large cytoplasmic granules with a core of medium to high electron density. A thin halo was usually present between the surrounding membrane and the dense core. The dense core, which almost completely filled the granules, was filamentous in texture and the granules measured 178–400 nm (mean 275 nm, see Fig. 3). The TG cells contained numerous microtubules and microfilaments and both free and membrane-bound ribosomes (Fig. 4). The ultrastructure of the TG cell is therefore quite distinct from that of gastrin-producing G cells and CCK-producing I cells, especially with respect to the morphology of the cytoplasmic granules.

As the distal half of the ileum of both pigs and monkeys contained many TG cells but very few gastrin and CCK cells, this part of the intestine was extracted in boiling water and/or acetic acid for radioimmunoassay[6]. As expected from immunocytochemical findings, only very low levels of immunoreactivity could be measured with gastrin-specific (As. 2604) and CCK-specific (As. 4698)[6] assays. Larger amounts of immunoreactivity were, however, detected by a COOH terminus-specific assay with As. 2609[6]. Gel chromatography of ileal extracts, monitored by CCK-, gastrin- and COOH-terminal-specific assays revealed low concentrations of immunoreactivity in positions corresponding to CCK-8, gastrin-34 and gastrin component I. Gastrin-17-like immunoreactivity was not detectable. In contrast, considerable amounts of immunoreactivity eluted in the position corresponding to the COOH-terminal tetrapeptide amide of gastrin (Fig. 5). This component has not yet been shown to be identical with the true COOH-terminal tetrapeptide amide, but in parallel studies on the porcine antropyloric mucosa (to be published elsewhere) strong evidence for the existence of this component was obtained.

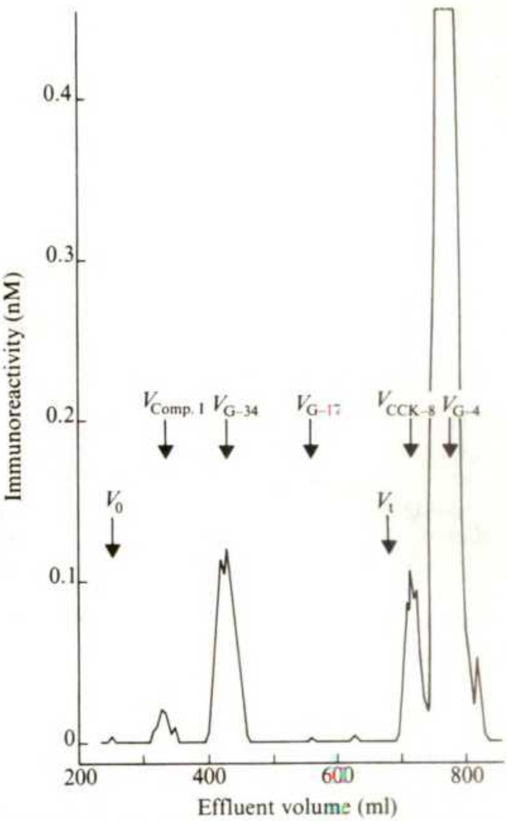

Fig. 5 Gel chromatography of a boiling-water extract of mucosa from the midsegment of the porcine ileum. 6 ml extract was applied to a Sephadex G-50 superfine column (25 × 2,000 mm) eluted at a flow rate of 20 ml h^{-1} with 0.25 M NH_4HCO_3, *p*H 8.2 at 4 °C. The column was calibrated with ^{125}I-albumin (void volume (V_0)), highly purified porcine gastrin component I ($V_{comp.I}$), gastrin-34 (V_{G34}), gastrin-17 (V_{G17}), CCK-8 (V_{CCK8}), highly purified synthetic gastrin tetrapeptide amide (V_{G4}) and ^{22}NaCl (total volume (V_t)). In addition to boiling water, midsegments of porcine ileum were also extracted (0.1–0.2 g mucosa ml^{-1}) with 0.5 M acetic acid, acetone (Merck), and acetone acidified with 0.1 M HCl (100:3, *p*H 1.0). However, when measured with an assay specific for the common COOH-terminal sequence of gastrin and CCK (antiserum 2609, also used to monitor the gel chromatography as shown) the latter procedures extracted <8% of the immunoreactivity measured in boiling-water extracts. Using boiling water extraction the following concentrations of immunoreactive gastrin-CCK-like peptides were found in mucosa from mid-ileum: Component I, ~0.2; gastrin-34, 2.3 ± 1.1; gastrin-17, <0.2; CCK-8, 14 ± 6; gastrin-4, 113 ± 7 (pmol per g mucosa wet weight; mean ± s.e.m., $n = 3$).

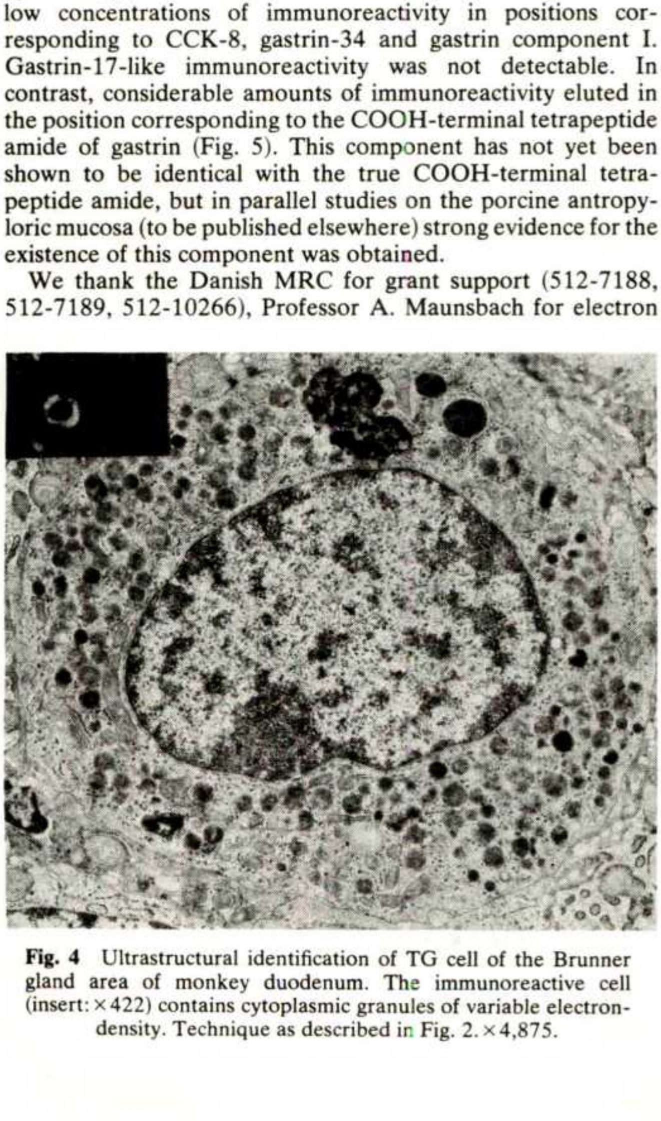

Fig. 4 Ultrastructural identification of TG cell of the Brunner gland area of monkey duodenum. The immunoreactive cell (insert: × 422) contains cytoplasmic granules of variable electron-density. Technique as described in Fig. 2. × 4,875.

We thank the Danish MRC for grant support (512-7188, 512-7189, 512-10266), Professor A. Maunsbach for electron microscope facilities, Mrs J. B. Lauridsen and Mrs E. Petersen for technical assistance and Drs J. H. Walsh, J. Morley and Professor V. Mutt for antibody 1295 and natural and synthetic peptides.

Note added in proof: Recently, Buchan *et al.*[9] described the ultrastructure of the intestinal gastrin cell. These small-granulated, D_1-like cells seem to be more numerous in the human than in the porcine or simian small intestines. Their ultrastructure is similar to that of small-granulated, gastrin immunoreactive cells described by us in human fetal duodenum[10] and in rat antropyloric mucosa (G_a cells[11]). Thus, the small intestines harbour three types of cells producing related peptide hormones: CCK (I) cells, COOH-terminal tetrapeptide amide (TG) cells and intestinal gastrin (GI) cells. The main cell type in the antrum is the G (gastrin) cell, but some species in addition contain small numbers of TG cells (monkeys) and G_a cells. The G_a cell may possibly be the murine equivalent of GI cells.

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Protein structural fluctuations during a period of 100 ps

A RECENT 9-ps molecular dynamics simulation[1] of the bovine pancreatic trypsin inhibitor (PTI) at 295 K revealed a rich variety of motional phenomena at the atomic level on a picosecond time scale. To obtain information about longer time processes, and to characterise more accurately the short time results, a 96-ps dynamical simulation of PTI at an average temperature of 306 K has been completed with the techniques used previously[1]; an extended equilibration period of 72 ps before simulation served to eliminate internal stresses. Analysis of the present simulation has confirmed most of the conclusions of the earlier study but has shown in addition that there are significant features of the dynamics that can be observed only over a longer period, ~100 ps.

An overall measure of the correspondence between the time-average dynamical and X-ray structures[2] is given by the radius of gyration. It equals 10.22 Å (with fluctuations of ±0.1 Å) for the former and 10.96 Å for the latter. The primary source of difference between the two are the positions of the charged external side chains, which may in fact have different average positions in solution and in the crystal[3,4]. The dynamics simulation also shows a strong coupling between the amino and carboxyl termini, in agreement with NMR solution results[5] but different from the crystal structure (see Fig. 1 of ref. 1). The core of the protein has a heavy-atom number density close to the X-ray value; within a radius of 8 Å from the protein centre, the calculated number density is 0.063 Å^{-3}, compared with 0.060 Å^{-3} for the X-ray structure.

Analysis of individual atom position fluctuations shows that, as a result of the more careful equilibration, they are slightly smaller than in the previous simulation. The average r.m.s. fluctuation for the α carbons is 0.60 Å (previously 0.74 Å) and that for all atoms 0.75 Å (previously 0.90 Å); the corresponding X-ray results from isotropic temperature factors are 0.68 Å and 0.74 Å, respectively (J. Deisenhofer, personal communication). The calculated mean-square positional fluctuations are found to be highly anisotropic for most of the atoms. For the α carbons, the overall averages are $\langle z^2\rangle = 0.252$ Å^2, $\langle x^2\rangle = 0.051$ Å^2 and $\langle y^2\rangle = 0.099$ Å^2, where a local principal axis system is used for each atom; in some cases, the largest component is more than 10 times the smallest. The differences in the orientations of the local systems for atoms in the same region, relative to a space-fixed axis system, make clear that the anisotropy is reflecting structural constraints rather than overall protein motion. Evaluation of the positional distribution functions shows that many of them differ significantly from the standard gaussian form, a consequence of the fact that the potential of mean force experienced by the atoms is not harmonic.

Because of their well-defined nature and possibility for direct experimental investigation, the aromatic side chain motions in PTI were studied in detail[6]. Tyrosine ring orientation fluctuations of ±30° from the average (r.m.s. value of 12°) were found for residues in the interior of the protein; this corresponds to a torsional potential of mean force considerably softer than that of the rigid protein. The time-dependence of the torsional motion approximates that expected from a Langevin equation for an angular oscillator with near critical damping; the calculated relaxation times for this motion are of the order of tenths of ps; for example, for Tyr 21 it is 0.2 ps. Comparison between the tyrosine side chain in a dipeptide and in the protein clearly demonstrates the importance of matrix damping effects due to the surrounding atoms in the latter.

An important result of the new simulation is the presence of long-lived fluctuations in the properties of PTI. Making use of 1-ps dynamic averages ('coarse-graining') to damp out rapid oscillations, it was found that fluctuations of 5–15 kcal mol^{-1} with a duration of 2–15-ps occur in the potential energy associated with the bond-stretching and bond-angle-bending coordinates, as well as with the non-bonded interactions. It seems that these energy fluctuations represent transitions of the protein from one potential minimum to another in the neighbourhood of the time-averaged structure. Direct evidence for this is given by the correlation of the potential energy fluctuations with transitions between minima of side chain dihedral angles and other internal coordinates.

A consequence of the infrequent structural transitions is that long periods of averaging are required to establish the fluctuations in global properties. Using the entire 96-ps period, we find the r.m.s. fluctuation in the kinetic energy to be 11.51 ± 0.1 kcal mol^{-1}, which yields a classical heat capacity of 3.14 ± 0.1k per protein atom (k is the Boltzmann constant). The previous simulation resulted in a smaller value for the r.m.s. kinetic energy fluctuation (9.3 kcal mol^{-1}) and a correspondingly smaller heat capacity (2.3 k per atom); test calculations show that such low values are due to the short time interval used for averaging. Apparently the heat capacity contains a contribution from equilibration among the different available states and so cannot be related as simply to models for protein dynamics as assumed in ref. 1. Long-lived fluctuations also occur in the atom positions, so that values obtained by averaging over less than 10 ps tend to be too low; for example, for the α carbon atoms, the r.m.s. fluctuation from 2-ps intervals are ~0.40 Å as compared with the value of 0.60 Å for the complete simulation.

The present calculation supports the earlier conclusion that a dynamic simulation can provide realistic and highly informative descriptions of the detailed atomic motions in the interior of a protein. The existence of large fluctuations in the structure and energy components indicates that the properties of proteins result from the occupation of a variety of thermally accessible states, even for times as short as 10–100 ps. It will be of interest to assess the role of these states in the biological activity of proteins and to determine the importance of alterations in the available states and transitions among them due to the solvent environment and ligand or substrate binding.

A full account of the extended simulation will be presented elsewhere. We thank John Ramsdell, Barry Olafson, and Thomas Jordan for their assistance and the NIH for support. J. A. McC. thanks the NIH for a postdoctoral fellowship.

Note added in proof: Frauenfelder, Petsko, and Tsernoglou (personal communication) have recently determined isotropic temperature factors for myoglobin at a series of temperatures and have used them to obtain atomic positional fluctuations; a dynamical simulation to compare with their results is in progress.

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reviews

Some pioneers of Natural History

Ray Desmond

The Naturalists: Pioneers of Natural History. By A. C. Jenkins. Pp. 200. (Hamish Hamilton: London, 1978.) £6.95.

MAN has always had some interest in animals and plants, if for no other reason than that they provide him with food. But curiosity rather than necessity compelled the attention of people like Aristotle who was one of the earliest scientific observers of nature. His *Historia animalium* shaped and directed scientific thought and enquiry for centuries.

Mr Jenkins's survey of the lives and achievements of some of the pioneers of Natural History begins with Aristotle and Pliny, and he pauses to consider mediaeval bestiaries and herbals before introducing the first encyclopaedic systematist, John Ray. Ray's skill and industry in classifying animals and plants earned him the apellation, "the Aristotle of England". But he was to be overshadowed by the Swedish naturalist, Carl Linnaeus, as the supreme classifier of the many forms of life. Linnaeus's sexual classification of plants, based upon the number of stamens a flower possessed, provided the professional and amateur botanist with a delightfully easy method of identifying plants, although one naturalist, Johann Siegesbäck of St Petersburg, rejected it as a "lewd and licentious system".

Both great and lowly have been devotees of the study of Nature. Mr Jenkins records that Frederick II, King of Sicily and Emperor of Germany, became a passionate naturalist through falconry and created a number of zoological gardens. He could also have mentioned the Moghul Emperor, Babur, who even during his conquest of India had time to observe a handsome plant or an unfamiliar animal.

Natural History has always provided a useful role for the amateur. Sir Joseph Banks, perhaps the most influential of all amateur naturalists, discovered its delights while a student at Oxford and after graduating persuaded his mother to rent a house at Chelsea so as to be near the Physic Garden of the Society of Apothecaries. It is said he paid £10,000 for the privilege of accompanying Captain Cook as naturalist on his first circumnavigation of the world. He encouraged Sir James Edward Smith, another enthusiastic amateur, to buy Linnaeus's collection of plants, insects, shells, minerals and manuscripts. He despatched gardeners to many parts of the known world to collect new plants for the King's gardens at Kew and Windsor.

Plant collectors were often explorers in their own right, travelling through unknown lands and risking their lives at the hands of hostile natives. Mr Jenkins praises the fortitude of David Douglas, a gardener at Glasgow Botanic Gardens, who was sent to North America in 1823 by the Horticultural Society of London to collect plants. Always ill at ease in society, Douglas was happiest on his own in the wilds of Canada where he endured incredible hardships in his pursuit of plants. The observation of Nature meant everything to him. There was the occasion when, despite extreme hunger, he would not allow a Franklin's goose which had been shot to be eaten because he wanted it for scientific purposes. He was only 35 when he was trampled to death by an enraged bull in an animal pit in Hawaii.

Not all naturalists led such adventurous lives. The French entomologist, Jean Henri Fabre, never left his native Provence, and the Reverend Gilbert White found fulfilment in his acute observation of natural things at Selborne in Hampshire. Indeed many clergymen escaped from the tedium of parochial duties through a study of botany, ornithology, entomology or some other branch of Natural History.

Natural History has its share of eccentrics and Charles Waterton, squire of Walton Hall in Yorkshire, is one of the most endearing of them all. He spent ten years building a high wall around his estate to protect wildlife from foxes, badgers and poachers. In his *Wanderings in South America*, he describes his vain attempts to persuade the vampire bat to suck his blood, and his bare-back ride on an alligator is a classic ancedote. As a devout Catholic he could not resist using his considerable skills in taxidermy to create animal caricatures of leading Protestants. He ridiculed Darwin's evolutionary theory by fashioning a simian-like creature from the skin of a red howler monkey and solemnly announcing it was the missing link in man's evolutionary chain.

Of course Charles Darwin had to be given a chapter in this book but the author spares a few pages for his fellow evolutionist, Alfred Russel Wallace, who meekly, almost masochistically, allowed himself to be eclipsed by Darwin.

Towards the end of his work Mr Jenkins admits that it "would more accurately be subtitled 'Some pioneers of Natural History' presenting as it does no more than a handful of naturalists". It is an easy matter to point to omissions. The two great bird painters, Audubon and Gould, are included but there is no mention of any eminent botanical artists such as Ehret and Redouté. In fact the zoological interests of the author predominate. Much of the author's life has been devoted to the cause of animal conservation and he informs us rather sadly that even Audubon indulged in the wanton killing of birds. He is an active supporter of zoos and discusses their role in protecting endangered species.

Mr Jenkins writes with enthusiasm and conviction but not always with accuracy. There is a sprinkling of mistakes, some of them clearly the result of careless proof-reading. The Linnean Society was founded in 1788 not 1778 (p46); William Harvey died in 1657 not 1677 (p192); the drawing of a lupin on p96 comes from the *Botanical Register* and not the *Botanic* (sic) *Magazine*; George III's mother, Princess Augusta, was never Queen (p88). Why are only floruit dates given for Nicholas Culpeper, John Gerard and William Turner (pp191–93) when the dates of birth or death are known?

Compared with Joseph Kastner's *A World of Naturalists* (John Murray: London, 1978) and David Allen's *Naturalist in Britain: a Social History* (Allen Lane: London, 1976), Mr Jenkins's account is lightweight indeed. However, with its generous and well chosen selection of plates, some in colour, it can be recommended as a pleasant introduction to the subject. □

Ray Desmond is Deputy Keeper at the India Office Library and Records, and a Past President of the Society for the Bibliography of Natural History.

Assessment of medical technology

The Image and the Reality: A Case Study of the Impacts of Medical Technology. By B. Stocking and S. L. Morrison. Pp. 80. (Oxford University Press: Oxford, London and New York, 1978.) Paperback £3.

THERE is no doubt about the public appeal of medical technology, as the achievement of a remarkable lady in Lancashire is raising nearly £1·5 million to buy and run a body scanner attests. Yet as the Director-General of WHO has recently said: "Technology for the sake of technology is a dangerous addiction-producing drug". Stocking and Morrison tackle the problems of technology assessment taking the body scanner as a case study.

The development of computed tomographic (CT) scanning of the brain by EMI, funded by the Department of Health and Social Security, was a remarkable British success story leading to clear improvements in diagnosis The same technique—X-rays passed through the body, detected by scintillation counters coupled to a computer—was then extended to whole body scanning. Whereas clinical evaluation had accompanied development of the brain scanner, in the case of whole body scanners evaluation was more complex because of the variety of organs involved and of alternative diagnostic techniques and they were extensively introduced before there had been thorough clinical evaluation The most rapid diffusion of body scanners has taken place in the USA (700 machines by 1977) and is largely attributable to their profitability, whereas in Britain diffusion has been much slower (11 machines by 1977) and for most of these the capital, but not running, costs have been met by charities rather than the health service.

The impact of body scanners is considered in this book on three broad levels: for patients, in terms of diagnosis, patient management and outcome; for the health service, in terms of the pattern and demand for care and for the professions involved; and for industry involved in manufacturing the equipment. As assessment of any technology must be concerned with the future, the authors discuss a variety of changes that may affect the contribution of CT scanners such as future developments of CT scanners, advances in other imaging techniques and in therapy, changes in cost and health service expenditure. This of course introduces many uncertainties but it is surely wise to try and see what may develop and the implications; as decisions affect the future it makes little sense to base them solely on an evaluation of the past.

In assessing medical technology, what is ultimately most important is the effect on outcome for the patient. Randomised controlled trials are common, if far from universal, practice in evaluating new drugs and therapies—however unusual such trials may be in assessing accepted techniques and therapies—and there is every reason to extend them to diagnostic procedures. Yet this is only just beginning.

Stocking and Morrison write· "The remarkable diffusion of [whole body scanners] prior to proper clinical evaluation is unsatisfactory". This is perhaps an understatement. The image of the health service promoted by the medical profession in Britain and many other countries is that it is crumbling from lack of resources and deprived of new developments that could be saving lives. What may be closer to the reality is that much of the medical profession is quite unscientific, indifferent to clinical evaluation and infatuated with technology and that there are ample resources if only they can be re-directed from activities which are useless and downright harmful into those where they may do some good.

This slim volume is interesting as a case study, and one can only lament that it is the first such study to approach a new technology in a systematic and comprehensive way. It is still more valuable in that it poses fundamental questions about the nature of health services and the impact of technology on these—which is not to doubt the highly beneficial effects in many cases. Finally, the authors make a positive proposal for a Medical Technology Assessment Group. With the prospect of almost limitless extensions of what *can* be done with medical technology, decisions as to what *should* be done can only become more pressing. The sooner Departments of Health and the World Health Organisation regard the assessment of medical technology as central to their functions the more hope there is of shaping health services to conform with the needs of human life

David Piachaud

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Testicular function

The Mammalian Testis By R. B. Setchell. Pp. 450. (Paul Elek: London, 1978.) £18.

THE testis is responsible for the production of spermatozoa and testicular hormones. Highlights of spermatozoa production include the initial mitotic divisions, the setting aside of stem cells which will re-initiate the spermatogenic process at some later date, meiosis, and finally, the dramatic metamorphosis of the prospective gamete that results in the formation of the acrosome, flagellum and mitochondrial helix and in the condensation of nuclear chromatin. Interestingly, many of these events occur only if the prospective gamete is sequestered behind the blood–testis barrier formed by the Sertoli–Sertoli occluding junctions bathed in fluids rich in steroid hormones and protected from noxious stimuli such as elevated temperature and radiation.

Dr Setchell's book deals with all the above aspects of testicular function. This is a formidable task because it requires that the author be knowledgeable in anatomy, physiology, biochemistry and endocrinology. For the most part, Dr Setchell achieves this difficult task with the result that the book is informative, clearly written, well illustrated and completely referenced. It will provide a valuable source of information for all those interested in testicular structure and function.

The chapters on vascular and nervous supply of the testis and scrotum, on fluid secretion and entry of substances into the seminiferous tubules, and on testicular thermoregulation are extremely good and reflect the author's long-standing research interest and expertise on these subjects. The chapters on spermatogenesis, endocrinology of the testis and endocrine control of the testis are presented in a thorough and professional manner. The chapters dealing with testicular metabolism and naturally occurring and induced dysfunctions of the testis are informative but not insightful. They consist primarily of a recitation of literature references and contain little or no synthesis of concepts Overall, this is an excellent book and Dr Setchell should be congratulated.

Larry Ewing

Larry Ewing is Professor and Head of the Division of Reproductive Biology in the Department of Population Dynamics, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland.

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Cooperative equilibria

Cooperative Equilibria in Physical Biochemistry. By D. Poland. Pp. 344. (Clarendon/Oxford University Press: Oxford, 1978.) £14.

COOPERATIVITY is an important phenomenon in molecular biology. It gives rise to sigmoidal binding curves, to allosterism, to steep conformational transitions, and plays a major role in assembly processes Quantitative treatments were developed for many simple systems and a general understanding was obtained for more complex ones. So far, the only summaries available were contained in several review articles and in a rather specialised book by Poland and Scheraga (*Theory of Helix Coil Transition in Biopolymers*, Academic, 1970)

The present book takes a more general approach. It starts from basic principles, gives a simplified presentation of the equilibrium theory and treats some specific systems as examples for its application. The book originated from a series of courses presented by the author to students at Johns Hopkins University at various levels of their curriculum It has the nature of a lecture script, is well written and reflects considerable teaching experience. The first chapter on simple multiple equilibria is rather elementary. In a long second chapter some concepts of statistical mechanics are repeated and some basic physical principles of intermolecular forces are presented. Three more specific and more demanding chapters are on transitions in linear biopolymers composed of single and multiple chains and on cooperative binding of ligands to linear macromolecules and to a two-dimensional lattice. A last chapter deals with the complications which are introduced when macromolecules are not composed of identical, repeating units but have a more complex sequence.

The main advantage of the book is that it may serve as a well written text for course work or for self-study. It lacks, however, a treatment of cooperativity in self assembly, a topic which is rather important and straightforward for linear systems like actin filaments or bacterial flagella. Two- or three-dimensional problems like the folding of globular proteins or transitions in membranes are not treated in any detail. Most importantly, I feel that a course on cooperative phenomena will be incomplete if the important kinetic features of cooperative systems are left out completely. My own teaching experience shows that equilibria and kinetics can be presented together with considerable advantage for a deeper understanding.

The author did not intend to present a systematic coverage of the research in the field of cooperative systems. The usefulness of the book as a review and as a source of references is therefore limited. References to the literature are few in number and incomplete. A rather severe disadvantage is the lack of a subject index. It has to be emphasised, however, that a short monograph like this can never satisfy all wishes and that the book will certainly be welcomed by many students and research workers who wish to be introduced into the field of cooperative equilibria.

Jürgen Engel

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Moorlands and montane grasslands

Production Ecology of British Moors and Montane Grasslands. Edited by O. W. Heal, D F. Perkins and W. M. Brown. Pp. 426. (Springer: Berlin, Heidelberg and New York, 1978.) DM108; $54.

THIS book comprises a series of research reports from the British part of the International Biological Programme (IBP) studies, mainly from Moor House in the northern Pennines and the sheep-grazed montane grasslands of Snowdonia. The overall emphasis is upon the development of ecosystem models for the two habitats based upon a variety of experimental studies of the component parts, together with mathematical models and simulations of certain critical processes, such as production, decomposition and peat accretion.

Much of the data from the Moor House IBP study has been published elsewhere, though it is useful to have much scattered data collated and condensed here The field studies of Smith and Forrest and the controlled environment work of Grace and Marks on the primary productivity of the blanket mire ecosystem have provided an interesting set of data from a generally neglected habitat. It is particularly useful to have in the same volume some comparable data from the southern English heaths (Chapman and Webb) and from the Cairngorm dwarf heath communities (Summers). Mean production values for the Moor House *Calluna/Eriophorum* blanket mire sites are about 250 gm^{-2} yr^{-1}, those from Dorset heaths about 320 gm^{-2} yr^{-1}, and from the Cairngorms come figures which vary considerably with such factors as soil fertility and degree of exposure, from 75–300 gm^{-2} yr^{-1}. Given the generally small sample areas studied, these figures are in very satisfactory agreement.

One advantage of publishing a collection of site orientated papers is the opportunity of putting together work which would otherwise be published in widely scattered disciplinary journals. So it is valuable to have the Moor House work on fauna and microbes in the same volume as the primary productivity work. A paper which will prove of particular value to peatland ecologists in general is that by Martin and Holding on the factors influencing microbial activity in blanket peat. Microbial activity was stimulated by adding an organic carbon source or by adding nitrogen, indicating nutrient limitations. The physical structure of the peat may restrict access to organic reserves. From the production and decomposition data, Jones and Gore construct a simulation model and Clymo develops a model of bog growth.

The Welsh grassland work is similar to that at Moor Mouse in that it deals with a montane habitat, but it differs in many other respects, such as the density of sheep. On Pennine blanket mire this was only 0.13 per hectare, whereas on the *Agrostis/Festuca* grasslands in Snowdonia it attained 9 per hectare. So the studies of sheep populations (Dale and Hughes) and their productivity (Brasher and Perkins) form important constituents of the Snowdonian work. The final summary of energy and nutrient flow patterns by Perkins is particularly useful and could have been emulated with profit by the Moor House team. From the diagram produced one can discern at a glance some fascinating aspects of the sheep-grazed ecosystem, such as the fact that there is more potassium in sheep dung (per unit area) than in sheep, and that earthworm nitrogen exceeds that in sheep whereas sheep have twice the energy content.

The bibliography is extensive and the book will prove an essential reference work to all who conduct research in these montane environments in Britain.

Peter D. Moore

Peter D. Moore is Senior Lecturer in Plant Sciences at King's College, University of London, UK.

Thin film devices

Active and Passive Thin Film Devices. Edited by T. J. Coutts. Pp 858. (Academic. New York, London and San Francisco, 1978.) £60.

If one expects the value of a technical work to keep pace with its cost and size then at a price of £60 at a weight of over 3 lb a purchaser is likely to be disappointed with the work under review. Unfortunately the subject matter is neither sufficiently thorough in its range nor adequately coordinated to constitute a satisfactory reference text on the chosen theme. How, you may ask, is this possible with 858 pages at the editor's disposal: the answer is that the editor has not adopted a policy towards his reader when choosing the subject matter. This is an applied physicist's work not an engineer's, and equal emphasis is given to realised devices as well as promising ones and failures, which might be acceptable if the reader were warned accordingly at the outset.

The book, containing thirteen chapters by specialists, covers thin film preparation, electrical properties of film materials and their uses or potential uses as passive elements and active components. The chapters describing solar cells, optical waveguides and magnetic and superconducting devices are useful reviews, but the references and appended notes indicate that the book was written by 1976. Nearly two years in printing is too long for such a rapidly moving field; consequently molecular beam epitaxy is given a proof reference and solar thermal converters which are in advanced development receive three lines of text.

The development history of thin film systems is littered with failures in deposition techniques and film materials, but such is the speed with which novel devices are invented that the search for new materials and growth processes continues unabated (except perhaps in the UK). Under such circumstances what should an editor include in a book on thin film devices? First, he must decide to whom the collected reviews are directed: the dust-cover suggests physicists, electronic and production engineers and material scientists. This is a broad readership that one cannot expect to have the discrimination of specialists when judging which devices are of practical value and which of historical interest. Thus the editor has given the same emphasis to thin film transistors and threshold switches (although their effective use after a decade is still awaited) as to resistors, capacitors, photoconductors, and so on. Of course one does not want to waste knowledge of physical characteristics arising from an unfulfilled product development but much of this could have been discussed in the chapters devoted to electrical properties of film materials. In view of the importance of silicon technology the most serious weakness perhaps is the omission of a chapter solely devoted to the growth and uses of epitaxial and amorphous silicon and of silicon compound films. Also there is an artificiality in restricting discussion to wholely thin film systems when thin films are so vital a part of most solid-state devices

When discussing the history of the use of thin films in electronics T. J. Coutts states that there would have been no advantage of using thin film resistors in the period of bulky valve circuitry, but the requirements of microminiaturisation made their use essential. This is an incomplete view of what occurred Thin film resistors using NiCr alloys came into use in World War II for waveguide attenuators and were later used for precision resistors The reason why vacuum deposited films were not used in valve circuits was that they broke down when operated at voltages in the 100V region However, development continued in the 1950s on transparent conducting coatings for windows and on the deposition of insulating films and the metallising of capacitor foils (which commenced before the War). Thus, considerable know-how existed on passive devices when the transistor became a production item and a more correct view is the convergence of technologies resulting in the manufacture of hybrid and integrated circuits.

In the chapter on "Preparation Methods for Thin Film Devices" the author has chosen to describe a variety of techniques rather than concentrate on specific kinds favoured for making the devices considered in the other chapters. The emphasis given to metal filaments and boats for evaporation suggests a subject treatment one would have found a decade or more ago and the availability of such refractory materials as B/Ti/N complexes for the high rate evaporation of aluminium is not mentioned Also magnetron sputtering is not described although it has helped to cause a renaissance in the use of sputtering in solid-state technology and most of the advances leading to cylindrical and planar magnetrons were made by 1974, that is, before the book was written.

The book is not an embracing text, and is inadequate in its treatment of deposition processes and lacks balance in its choice of devices. However, as stated above, there are several specialist chapters which are valuable reviews

L. Holland

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Reflection seismology

Reflection Seismology: A Tool for Energy Resource Exploration. By K. H. Waters Pp 377. (Wiley: New York and Chichester, UK, 1978.) £19.75.

By far the most successful method of determining the structure of the upper few kilometres of the Earths' crust has been and continues to be the art and science of reflection seismology. This book provides an essentially complete survey of the subject from the basic physical principles to the multitude of data generation and analysis tools currently used by the oil exploration industry. The volume is perhaps too short on mathematics. However, the physics, including the underlying assumptions of each technique, are generally well presented.

The volume begins with a general description of the pertinent aspects of elastic wave propagation in the upper crust of the Earth Following chapters cover seismic energy generation techniques, data-gathering methods, stacking and filtering techniques, and the reduction of processed data to the geometry and physical properties of the rock formations below. Introductions to such recent and current topics as homomorphic deconvolution, adaptive filtering, maximum entropy spectral analysis, integral and differential wave equation migration, shear waves, high frequency methods and three-dimensional data collection and analysis are included.

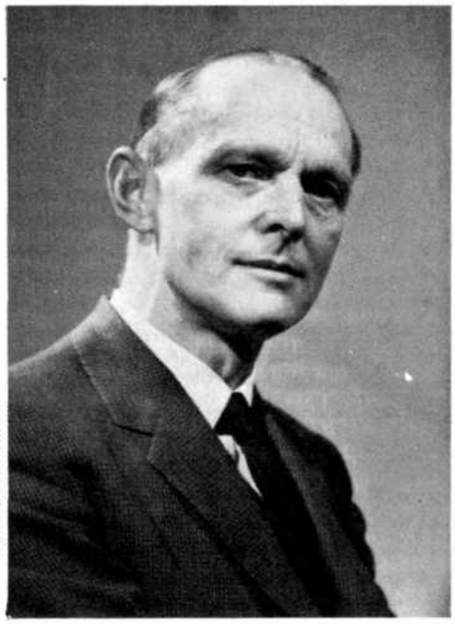
The professional exploration seismologist will find that they know a great deal more about many of the subjects covered in this book, and students whose desire is a thorough knowledge of any given topic will require a more detailed and explanatory presentation However, the volume is well referenced (up to about 1973). The book is appropriate and recommended for those who want a basic technical introduction to exploration seismology from beginning to end.

Tom Landers

Tom Landers is a staff member at the Applied Seismology Group of Lincoln Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts

obituary

Norman Feather, 1904-1978


John Dewar Studios

Professor Norman Feather, FRS, died on 14 August 1978 at the age of 73. His death has removed one of the last of that brilliant group of young men who gathered round Rutherford at the Cavendish Laboratory, and later guided the postwar expansion and flowering of academic physics in Britain.

Feather was born at Crimsworth, in the West Riding, the son of two school teachers. At Bridlington School he came under the influence of an inspiring science master, and this led him to decide to study physics at Trinity College, Cambridge. In 1926 Feather was awarded his BA degree at Cambridge, and the BSc degree of the University of London.

For the next three years he worked as a research student, under Chadwick, on the development of cloud-chamber techniques and their application to a study of the rare long-range α-particles from Radium-C. In 1929 he was awarded a Fellowship of Trinity College. The award was for a period of four years, and he spent the first of these years at Johns Hopkins University in Baltimore, at the behest of R. W. Wood. He returned to Trinity College in 1930 and submitted—successfully—his PhD thesis.

In his luggage Feather had brought back with him 300 'dead' radon bulbs, from the Kelly Hospital in Baltimore, containing in all about 100 millicuries of polonium. It was with this polonium that Chadwick, Dee, and Feather established in 1932 the production of neutrons when beryllium was bombarded by α-particles, and in particular Feather, using his automatic cloud-chamber, produced the first clear evidence for the transmutation of nuclei by neutrons.

Feather's next cloud-chamber experiment was a study, jointly with Bretscher, of the photo-disintegration of the deuteron. The value they obtained for the deuteron binding energy was challenged by Bethe, on the basis of his theoretical studies of the rate of energy loss of charged particles in matter. The Bretscher–Feather value was subsequently vindicated by more refined experiments elsewhere. Feather had never doubted that it would be: his calibration data had been accumulated during years of painstaking observation and comparison.

Feather frequently pointed out to his colleagues and juniors that he had never been exposed to the higher reaches of mathematics nor to any quantum theory—'just a little applied mathematics'. And he would go on to maintain, with a glint in his eye, that there were still things to do in nuclear physics where insight and intuition were the best guides. When it was necessary his mathematics tended to rise to the occasion, as in his early studies of the statistics of the prompt- and delayed-coincidence techniques which he used in β-ray spectrometry and the investigation of nuclear isomerism, in the late 1930s. But when his insight and intuition were involved they were backed up by a vast and detailed knowledge of nuclear systematics, which found expression in his monograph on *Nuclear Stability Rules* (1952) and in his later writings on nuclear fission.

When Chadwick went to the Chair of Physics in Liverpool in 1935 Feather went with him, but remained there for only one year, during which he wrote his first undergraduate textbook, *An Introduction to Nuclear Physics*. In 1936 he returned to a lectureship in Cambridge, in charge of his own research group. A year later Rutherford died, and subsequently Feather found himself with more responsibility for the nuclear work in the Cavendish Laboratory. During the war years he carried a very heavy administrative load, both in the teaching programme and in the war-time work on nuclear fission.

In 1945 Feather was elected to the Fellowship of the Royal Society, and appointed to the Chair of Natural Philosophy in Edinburgh. He occupied this chair, as Head of Department, for 30 years. His first task in Edinburgh was to build up a department in which the techniques which had transformed nuclear experimentation during the war years could be developed and exploited. He was joined by a number of talented young men, and work was soon under way in the β and γ-ray spectrometry of (mainly) naturally radioactive species, and in radiation chemistry and fast-neutron physics. Feather was not a man to stifle or constrain those of his staff who wanted to work in other fields, and projects on cosmic rays, meteorology, fluid dynamics and metal physics got under way too. The undergraduate curriculum was drastically overhauled, and by his own example Feather transmitted his view of the interdependence of undergraduate teaching, postgraduate training, and research.

With his Department running smoothly, Feather for a time concentrated his energies on writing and produced a series of texts aimed at undergraduates. Although his rather mannered style was not altogether congenial to the undergraduates of the 1950s and 1960s, the combination in these books of care for language, extensive knowledge, and a scholarly and disciplined approach, was an affirmation of values which the author felt impelled to exhibit to his students.

In the 1960s Feather's attention turned again to the fission process, and a series of papers followed which dealt with the process of ternary fission, and especially the α-particles emitted in fission. His lifelong interest in the various aspects of α-emission continued into his retirement. His penultimate paper, published in 1978, discussed the two solutions that have been proposed to the problem of the polonium haloes in mica. Something of the essence of the man flashes out in a characteristic sentence: 'Because these two suggestions appear to exhaust the logical possibilities of explanation, it is tempting to admit that one of them must be basically correct, but whoever would make this admission must be fortified by credulity of a high order.'

Feather's keen and incisive mind and his remarkable detachment made him a distinguished academic administrator.

His services to such bodies as the Scottish Universities Entrance Board, the Home Defence Scientific Organization, and the Royal Society of Edinburgh (which he served for many years, as Councillor, as General Secretary, and then as President) were generously given and widely appreciated. The University of Edinburgh acknowledged his notable services to the University community by the award of the Honorary Degree of Doctor of Laws, just a few weeks after he retired.

R. M. Sillitto

Herbert Dingle

THE well-known spectroscopist, astrophysicist, philosopher of science and critic of relativity, Herbert Dingle, died in Hull on 4 September 1978, not long after his 88th birthday.

Although of Cornish descent, Dingle was born in London, close to his beloved Surrey county cricket ground, the Oval, on 2 August, 1890. He spent most of his boyhood and youth in Plymouth, where his mother had returned on the death of her husband. After leaving school at the age of fourteen, Dingle worked for over ten years as a clerk by day and studied, as best he could, by night. In 1915 he won a Royal Scholarship for Physics at Imperial College. He graduated in 1918, having already been appointed to the staff of his department. The same year he married Alice Westacott. She predeceased him by over thirty years. He remained devoted to her memory. They had one son who became an industrial chemist, but he too died before his father.

More than three-quarters of Dingle's academic career was spent at Imperial College, where he was eventually promoted to a chair in natural philosophy in 1937. Originally, he specialised in spectroscopy under Alfred Fowler, the successor of Norman Lockyer. On Fowler's retirement in 1935, he became head of the spectroscopy section. Under Fowler, Imperial College had been in the forefront of physics, in the great days of line spectroscopy following the introduction of the Bohr atom. It was no fault of Dingle's that his time as head of the spectroscopy section coincided with a world-wide lull in the subject, prior to its revival, under the impact of nuclear physics, in the nineteen-fifties. Nevertheless, Dingle did useful work in spectrochemical analysis and pioneer research on the spectra of fluorine II and III. He wrote much on the laboratory and astronomical applications of spectroscopy.

His book *Modern Astrophysics*, published in 1924, was one of the first on that subject. He was also part author of *The Splendour of the Heavens*, published in 1923, and *The Life and Work of Sir Norman Lockyer*, published in 1929.

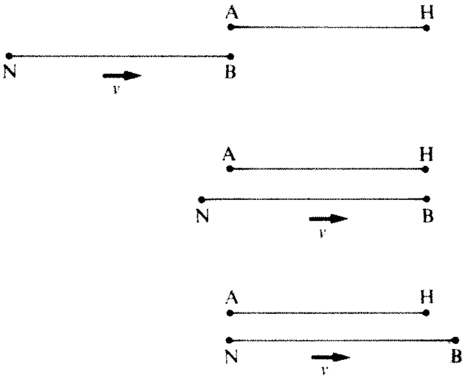
From the early nineteen-twenties Dingle was actively concerned with the philosophical as well as with the practical aspects of physics. He developed a highly professional interest in the philosophy of science and as a writer in that field he became very well known, particularly in the United States, which he visited for two lengthy periods in the thirties, the second time as Lowell Lecturer at Harvard. During the prolonged absence of Sir George Thomson from Imperial College during World War II Dingle was effectively in charge of the physics department.

Dingle's philosophy of science, as expounded in his books *Science and Human Experience* (1931) and *Through Science to Philosophy* (1937), the latter based on his Lowell Lectures, was in the new unfashionable tradition of British empiricism. He believed that the essential basis of science was the rational correlation of elementary experiences which can be made objective. This led him to adopt a philosophy which had much in common with that of Ernst Mach.

In 1946 Dingle was appointed Professor and Head of the Department of History and Philosophy of Science at University College, London, a post that he held until his retirement in 1955. Although no longer active in spectroscopy and astrophysics, he was elected President of the Royal Astronomical Society from 1951 to 1953. He was also President of Commission 41 (History of Astronomy) of the International Astronomical Union for some years and was a Vice-President of the International Union for the History of Science from 1953 to 1956. He founded in 1948 the Philosophy of Science Group of the British Society for the History of Science (which later became the British Society for the Philosophy of Science). Two years later, with the support of the late Dr H. P. Morrison of the publishing firm Nelson, he founded *The British Journal for the Philosophy of Science*. From 1955 to 1957 he was President of the British Society for the History of Science.

In the latter year he began his famous campaign against the special theory of relativity which dominated the last twenty years of his life. He had originally been sympathetic to the theory, particularly because of the ostensibly 'operational' philosophy that had guided Einstein in its creation, and in 1940 he published a Methuen monograph on it. His doubts originated when, while reading Sir George Thomson's *The Foreseeable Future*, he came across a passage relating to the 'clock paradox'. As a result, he engaged in a controversy in the correspondence columns of *Nature* with Professor W. H. McCrea, FRS, which led to a tremendous resurgence of interest in the 'paradox' and a spate of publications on it.

Later Dingle attacked the entire concept of relativistic time. His basic objection was that, according to the theory, two similar clocks, A and B, in uniform relative motion V work at different rates, but since the relation of each to the other is symmetrical it follows that, if A works faster then B, B must work faster than A. He argued that this was a contradiction and so the theory must be false. The point at issue was a subtle one and concerned the way in which Dingle used a particular concept which he called 'rate of clock'. He exemplified this by taking A and B to be together at epoch zero according to each and introducing two other clocks H and N at rest relative to A and B, respectively, but not spatially coincident with them. He compared the times t_1, t'_1 assigned to the epoch of B's momentary coincidence with H by the observers using the clocks A, B respectively, and the corresponding times t_2, t'_2 assigned to the epoch of A's momentary coincidence with N. He then argued that, since special relativity led to the formulae, $t_1=\beta t'_1$, $t_2'=\beta t_2$, where β is the reciprocal of $\sqrt{(1-V^2/c^2)}$, a contradiction results. This is because in the former case the ratio of the 'rates' of clocks A and B is given by t_1/t'_1 and in the latter case by t_2/t'_2, and hence this ratio has two incompatible values.



The difficulty can be resolved by rejecting Dingle's assumption that ratios such as t_1/t'_1 determine invariant relative 'rates' of clocks A and B. His implicit requirement that the epochs assigned to *any* event by A and B, respectively, should always be in the same ratio would imply that by a new choice of time unit for one of these clocks it could be arranged that the times assigned to any given event by A and B would be the same. Dingle's requirement is therefore equivalent to adopting the Newtonian concept of universal time, and this is incompatible with special relativity.

Dingle believed that his question concerning clock-rates was not answered but evaded by his critics. In his struggle with what he came to regard as the 'relativistic establishment', described in his book *Science at the Crossroads* (1972), he was often treated in a way that convinced him that truth was being sacrificed to dogma. His attitude towards relativistic physics was not, however, purely negative, for he adopted, and spent much time in developing, the alternative 'ballistic' theory of light-transmission due to W. Ritz.

Despite his criticism of special relativity, Dingle never lost his respect for Einstein's genius, just as he no less admired his fellow quaker Eddington despite submitting the latter's philosophy of physics to devastating criticism in his brilliant Eddington Memorial Lecture on *The sources of Eddington's philosophy* (1954).

No obituary notice of Dingle should fail to mention the beautiful literary style that graced all he wrote. He had a deep knowledge and appreciation of English literature, particularly poetry. Two of his books were on *Science and literary criticism* (1949) and *The mind of Emily Brontë* (1974). His friends will long remember his lively conversation spiced with a rapier-like wit and numerous anecdotes, and his many acts of kindness, not least to those refugees from Europe whom he and his wife so generously assisted in the nineteen-thirties.

G. J. Whitrow

M.H.L. Pirenne

Maurice Henri Léonard Pirenne, well known for his work in the physiology of vision, died in Oxford on 11 October 1978.

Maurice Pirenne was born in Verviers, Belgium in 1912. He graduated DrSc at Liège, and spent the next year doing research in molecular physics with Professor Debye. The following three years were spent at Columbia University, New York, with Selig Hecht studying the biophysics of vision, and it was probably this period, more than any other, that determined his future interests.

In 1942, a classical paper by Hecht, Shlaer and Pirenne demonstrated that the uncertainty of seeing near the visual threshold, usually attributed to biological variation, was in fact largely accounted for by purely physical variations in the small number of light quanta absorbed by the visual photopigment. Much of Pirenne's later work was concerned with the visual threshold and its relationship to visual acuity, ranging in scope from the practical problem of screening servicemen for night blindness, to neurophysiological studies of the thresholds of 'on' and 'off' units and their interaction.

He returned to England in 1941 and worked in Cambridge, London and Aberdeen before joining the University Laboratory of Physiology, Oxford, in 1955. He became a fellow of Wolfson College, and worked in Oxford until his retirement. As a teacher, he will be remembered particularly for his demonstrations and class work. The experiments that brought the subject so vividly to life were based upon long hours of preparation and adjustment.

Maurice Pirenne was the son of an artist, and while he was still at school his father encouraged him to read the work of Brücke and Helmholtz on vision and art. His interest in drawing and painting lasted all his life, and in recent years he had returned to the study of the relationship between visual physiology and the perception of pictures. *Optics, Painting and Photography* (1970) is concerned mainly with perspective, and how far a painting in perspective can really look convincing to an observer who, instead of viewing it with one eye placed in the ideal position, uses both eyes and moves to different positions. His last publication, in 1975, was on Vision and Art.

All Pirenne's scientific work was characterised by acute commonsense and painstaking accuracy. The same qualities appear in his published work. *Vision and the Eye*, which first appeared in 1948 remains the clearest and most readable introduction to the subject. He was internationally recognised as an authority on visual physiology, and in 1972 received an ScD at Cambridge and was appointed a Foreign Member of the Royal Belgian Academy of Sciences.

F. H. C. Marriott

B. B. Roberts

Brian Birley Roberts, authority on polar problems and ornithologist, died in Cambridge on 9 October 1978 at the age of 65. For thirty years Head of the Polar Regions Section, Foreign and Commonwealth Office, he was also a Research Fellow at the Scott Polar Research Institute, Cambridge and a Fellow of Churchill College.

His interests in the polar regions started when he was still a schoolboy and he became probably the foremost specialist in the world. As an undergraduate at Emmanuel College, Cambridge, he led expeditions to Iceland and East Greenland in 1932 and 1933 and later joined the British Graham-land Expedition (1934–1937). As an ornithologist, he will be particularly remembered for his paper on Wilson's petrel published in 1940, which has stood the test of time as a good and careful study. It dealt with breeding behaviour, growth and reproductive success; the remarkably long annual migration was illustrated by monthly distribution maps and supported by ringing returns, which proved that individuals return each year to the same mate and burrow in the Antarctic.

In 1940 he took a Cambridge PhD and was awarded the Polar Medal and the Bruce Memorial Prize of the Royal Society of Edinburgh. He received the Back Award of the Royal Geographical Society in 1948. During the war years he served in Naval Intelligence and advised the War Office on cold climate equipment. He revisited the Antarctic in 1950–51, 1960–61, 1964, 1972 and finally in 1976, shortly after his retirement.

Brian Roberts played a formative role in discussions leading to the Antarctic Treaty (1959) which ensured the continuance of the freedom of scientific investigation and the free exchange of results which marked the International Geophysical Year 1957–1958. He did much to promote the Agreed Measures for the Conservation of Antarctic Flora and Fauna (1964) and the Convention for the Conservation of Antarctic Seals (1972). While he asserted that he had had to *eat* more seals than any others present, he was dedicated to conservation and also regarded that convention as the forerunner of a broader convention dealing with other Antarctic marine living resources, especially krill, now being negotiated. Although often the only scientist in a group of diplomats and lawyers, it was to Brian Roberts that they frequently turned for help in resolving a diplomatic impasse.

He played an active role in the formation of the British Antarctic Survey and he had an enduring influence on the development of the library of the Scott Polar Research Institute and the establishment of what developed into the International Glaciological Society. He was a leading expert on Antarctic history, terminology and place names.

He was made CMG in 1969 and in 1976 was awarded the Founder's Medal of the Royal Geographical Society. He was president of the Antarctic Club in 1963 and of the Arctic Club in 1973.

Many senior polar scientists benefited, as young research workers and later, from the stimulation of his presence, his phenomenal range of detailed knowledge and constructive criticism. His beaming smile, kindness, wit, scholarship, experience and great capacity for work will be long remembered by his many friends, who will find it difficult to believe he is no longer there.

R. M. Laws

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AHMAD, F., **SCHULTZ**, J., **RUSSELL**, T. R., and **WERNER**, R. (edited by). Differentiation and Development. (Miami Winter Symposia, Vol. 15.) Pp.xxii + 533. ISBN-0-12-045450-5. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) $29.50; £19.15.

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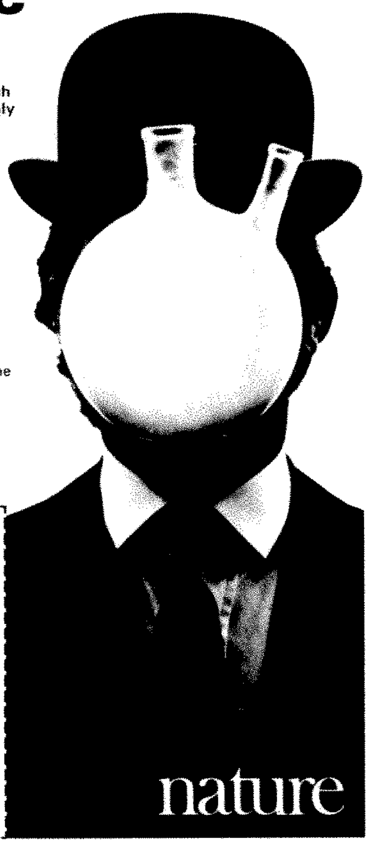

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what's new—lab design and safety

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A feature on the 1979 FASEB Exhibition will appear in the 29 March issue.

Dohm. Dangerous liquids can be transferred safely and quickly without waste with the polyethylene syphons produced by Dohm London Ltd. The AA model has a 3/4 pint bellows capacity, can provide a flow of up to 1½ gallons per min and is 91 inches in overall length. The AAG is of 3 pint capacity, flow rate 3 gallons per min and overall length 128 inches. The Dohm safety inspection lamp is suitable for use in confined spaces: the light is evenly diffused by an anti-glaze reflector to prevent eye strain. For situations in which 110 or 240 V power supplies are a safety hazard Dohm produce the DOHMplug range. These compact transformer/rectifier units give a 40 W output at 6, 12, 25, 50 and 110 V.

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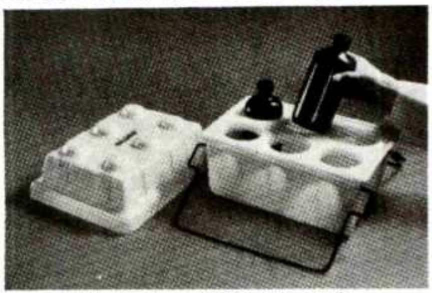
The Nalgene 6505 bottle carrier

Sintacel. Metriscope from Sintacel is a comprehensive range of equipment capable of fulfillng the servicing and furnishing requirements of educational and laboratory accommodation. The range provides non-fixed furniture with overhead service booms and drainage equipment. All furniture is either movable or mobile and the working surfaces, with their flexible service/drainage connections, can be quickly re-arranged. The overhead service booms may provide such services as gas/electricity/water/vacuum/lighting. Drainage is normally dealt with by means of a regularly spaced grid of floor outlet points, but if so required, wall or overhead drainage may be provided.

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Nalgene. An extensive range of safety equipment suitable for laboratory use is available from the Nalge Division of Sybron Corporation. The Nalgene emergency eyewash station weighs only 11 pounds and is based on a reservoir of 2.5 litre volume and once the release bar is depressed a continuous stream of water is produced: both hands are left free to keep the eye open to receive the stream, which will last from four to six minutes. The 6355 range of Nalgene safety shields are formed from 0.188-inch Lexan and can resist impact from explosions, flash fires, flying particles or splashing liquids. These shields are mounted on a heavy epoxy-coated steel base to ensure stability. Nalgene 6355 face shields are made with 0.06-inch polycarbonate. The transparent shields cover the front, sides, and top of the head, plus the front of the neck. For the transportation of hazardous substances around the laboratory Nalge provide two types of carrier. The 6501 safety bottle carrier is made of polyethylene and holds a 5-pint or 1-gallon glass bottle securely with provision for a snap-on cover. The 6505 bottle carrier holds up to six 1-pint glass bottles in separate compartments. Large volumes of liquids can be safely stored in Nalgene carbouys. Also available from Nalgene are safety wash bottles. These are intended for use with inflammable or toxic substances and are made of red polypropylene.

Circle No. 58 on Reader Enquiry Card.

The emergency eyewash station from Nalge

Data Acquisition. The DA 1500 mercury vapour detector is manufactured by Data Acquisition Ltd, in association with ICI Ltd., Mond Divi- principles based on the absorption of sion. The instrument operates on ultraviolet light by mercury at a wavelength of 2,537 Å. The basic systems comprises a sample cell fitted with a UV light source and measuring and reference detectors. The detector output signals are fed to a solid state amplifier whose linear output, drives a four digit display. An output is also available to drive a recorder via front panel terminals. The instrument is portable and measures approximately 160 × 160 × 700 mm and weighs about 12 kg.

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The DA 1500 Hg vapour detector

Berthold. The advances made by Berthold, in large area contamination probes, have been applied to the new whole body contamination monitor. This allows the simultaneous monitoring of the face, clothing (at the front of the body to the feet), soles of feet and hands. All the detectors are the new xenon filled type having very high sensitivity for iodine-125 and carbon-14 and without the requirement for a continuous supply of gas to the detectors. The device routinely stores background information when not in use and automatically subtracts this at the appropriate rate during measurement. The system permits the measurement of most transuranium isotopes through their emitted K-radiation thus allowing them to be monitored despite the unavoidable distance between the detector and the body to be checked. For institutions where exposure to high activity radiation is possible, the monitoring of workers normally needs to be recorded and stored. The system can be supplied and delivered with an identity card reader with data output to either a Teletype or on-line computer.

Circle No. 56 on Reader Enquiry Card.

Research Products International. The RPI brochure 'Contamination and Radiation Monitoring Instruments' details the RPI line of protable geiger-muller tube and sodium iodide crystal instruments. These instruments are used for the detection and monitoring of α, β, γ and X ray radiation in research, hospital, teaching and industrial laboratories. Rugged dependability and minimal maintenance are provided by solid state components mounted on glass reinforced printed circuit boards. Advance circuitry stabilises battery voltage, affording consistent accuracy throughout battery life without need for recalibration.

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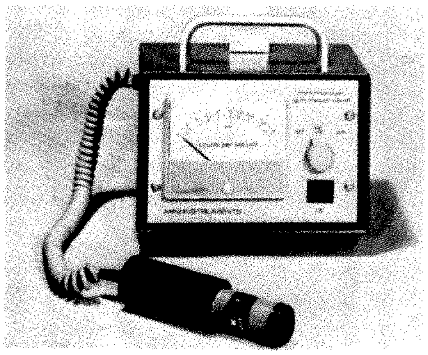

The RPI model 5-10X contamination monitor

Safety Technology Ltd. A new concept in Zener safety barrier systems for electronic interfaces has been announced by Safety Technology Limited of Feltham, Middlesex. The new system, designated Eurobloc-80, uses specially designed Zener barriers called Euroblocs, which 'snap' into internationally recognised terminal block rails within equipment cabinets. Although electrically identical to existing types of Zener barriers, the new Euroblocs are built into standard terminal blocks, which clip into the DIN rails with a high packing density. The connection and replacement of each Eurobloc can therefore be made very quickly, and arrays will take less space. STL claim that it is possible to obtain up to 3 times the packing density of conventional barriers.

The old type of barrier required specially drilled busbars and a prolonged assembly process involving the tightening of 4 lock-nuts per unit. In contrast, each Eurobloc clips straight onto the standard DIN terminal rail and requires no additional mechanical fixing. Electrical connectors are also easier, as each Eurobloc will accept basic stripped and tinned leads—the old barrier needed special ring tags. A further inherent advantage of the new system is the ability to make all connections at the front face of the module. It is no longer necessary to have either raised busbars or rear access for mounting purposes. Dimensionally the Eurobloc barrier measures 58 × 67 × 18 mm—effectively, half the size of the conventional unit. Initially ten standard types of Eurobloc are being introduced, covering the operating voltages of 4, 10, 15, 22 and 28V, in positive or negative polarity. Double barrier forms may be constructed by simply 'doubling-up' Euroblocs. a.c. voltages are catered for by using a special a.c. adapter. The ten standard Euroblocs can thus be applied in over 60 different variations for which BASEEFA approval has been obtained. Identification is achieved using colour coded labels attached to the Eurobloc.

The use of standard 'building bricks' —the DIN standard terminal block— means that costs are minimised. Wiring is generally neater and cabinet space, which is expensive in environments like oil production platforms, reduced.

The Eurobloc 80 range has been BASEEFA Certified to the New European Certification CENELEC EN.50-020 and UK Certification BS 5501 Part 7. It thus fully complies with the requirements of intrinsically Safe barriers for use in hazardous areas.

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Variable Volumetrics. Variable Volumetrics introduce a disposable transfer pipette with ultra lean tube. This polyethylene transfer pipette has a tube so narrow that it will fit into almost any size test tube. It is a one-piece pipette complete with its own bulb, and will eliminate the danger of broken glass, operator contamination from having to change rubber bulbs and cross contamination of samples with contaminated pipettes or rubber bulbs. The pipette delivers 21–24 drops per ml, and has a maximum capacity of 3 ml.

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Gallenkamp. A combination of traditional and modern timber-based materials, Gallenkamp System M furniture is robust, hard-wearing and attractive in appearance. The modular units are versatile and freely interchangeable. Most of the units are designed so that they can be packed flat. Modules available incorporate benches with a choice of drawer, cupboard or tray under storage units, sinks, mobile demonstration bench units and a fume cupboard.

Circle No. 52 on Reader Enquiry Card.

Jencons. A mitten style pyroglove has been added to the range of safety gloves marketed by Jencons (Scientific) Ltd. This mitten style is available in wrist or gauntlet length, and consists of a separate thumb sleeve and an area for the four fingers. Normal style Pyrogloves in wrist or gauntlet length are also available and were made to replace the asbestos type gloves previously used by glassworkers when handling their hot glassware direct from the flame or annealing oven, yet possess the tactile qualities of ordinary leather, will absorb moisture and retain water vapour permeability—thus making them comfortable in use.

Circle No. 51 on Reader Enquiry Card.

Becton Dickinson. BBL Biohazard cabinets from Becton Dickinson are officially listed under National Sanitation Foundation Standard 49 and are Class II Type A or Type B containment hoods intended for work involving low to moderate risk agents, such as aetiologic agents CDC Classes 1–3, low to moderate risk oncogenic viruses, recombinant DNA and other uses where National Sanitation Foundation Standard 49 design specifications apply. The cabinets are available in 47- and 70-inch sizes. A vertical laminar air flow incorporates HEPA filters with minimum collection efficiencies of 99.97% for 0.3 μm and larger particles, which minimises cross-contamination within work area by inhibiting particles from moving horizontally. The complete cabinet is at negative pressure to the laboratory atmosphere.

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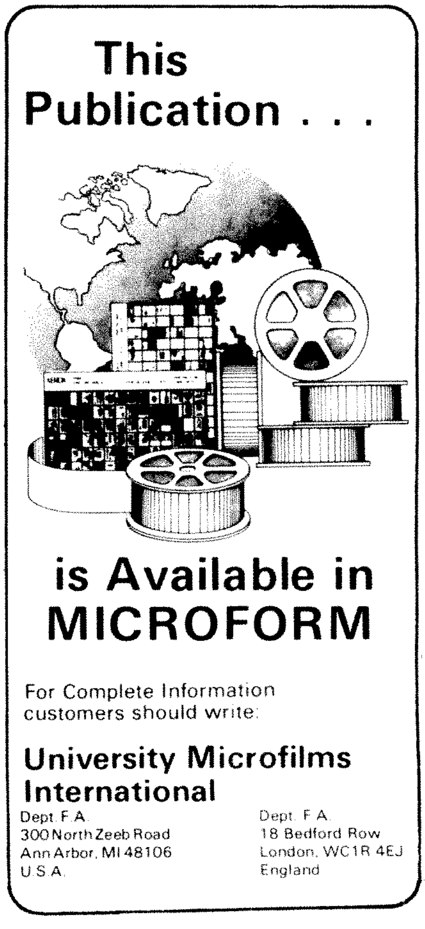

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Circle No. 39 on Reader Enquiry Card.

Temperature gradient incubator. Scientific Industries. The Scientific Industries temperature gradient incubator allows 60 different samples to be incubated simultaneously at 30 different temperatures in a single self-contained precision instrument. The incubator allows aerobic and anaerobic measurements, accommodating a variety of media which may be incubated at the same time or independently. 60 liquid samples can be incubated in glass L-tubes, optically selected to permit nephelometric or colorimetric measurements without sample transfer. There is also a built-in shaker. Aerobic organisms on solid media can be incubated in parallel trays.
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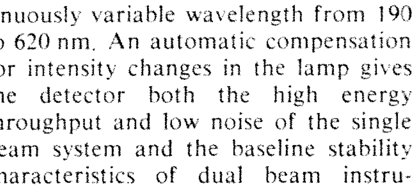

Scientific Industries incubator

Variable wavelength absorbance monitor. Isco. The Isco model 1800 chromatographic monitor provides continuously variable wavelength from 190 to 620 nm. An automatic compensation for intensity changes in the lamp gives the detector both the high energy throughput and low noise of the single beam system and the baseline stability characteristics of dual beam instruments. Easily removable flow cells allow the instrument to be used on HPLC systems, as well as in low pressure column chromatography. The instrument may be used in a cold room at 5 °C with no precautions.
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Recorder. Bryans Southern. The Bryans BS 8000 is a multichannel recorder with data processing and storage facilities. The BS 8000 enables one to digitise, record and process up to 8 channels of analogue data. Raw or processed data are plotted in real time on the XY plotter or stored on a flexible disk. Each flexible disk is equivalent to 100 yd of chart paper, yet any sequence of data can be retrieved in graphical form in a few seconds.
Circle No. 42 on Reader Enquiry Card.

Anti-mouse IgD antibody. Sera-Lab announces the availability of a monoclonal antibody against mouse IgD-like antigen. The determinant molecule appears to be a tetramer of 185,000 consisting of two units of 70,000 and two of 22,500 molecular weight. This addition to the range of monoclonal antibodies seems to recognise the Ig-5 locus regardless of H-2 haplotype. The antibody is suitable for use in complement-dependent lysis tests as well as direct reactions using fluorochrome or isotope markers. Because of its high titre, which approaches 10^6, the antibody is an ideal reagent for the study of the function and structure of murine IgD-like molecules. The exact allotype specificity, using the Goding nomenclature, has been defined as Ig5.C3(H6/31).
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Wet test meter. International Gas Apparatus. A portable meter capable of accurately measuring small volumes and low flow rates using the traditional wet test meter principle, is now available from International Gas Apparatus. A special feature of the meter is its capability to operate up to 30 p.s.i. working pressure. Measurement of gas volume is better than 0.5% and that of liquid temperature ± 1% of full scale over a temperature range of 0–50 °C.
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Small scale protein concentrator. Sartorius. The microcollodion bag from Sartorius enables a 250 times concentration of urine, CSF or aqueous humour to be carried out in only 3 min. The 1-ml sample is filtered under pressure in the 8-nm pore bag which has retention efficiency of 100% for cytochrome *c*.
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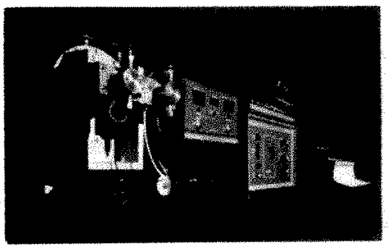

HPLC range. Pye Unicam. The LC-XP Series, a new range advanced, high performance liquid chromatography instruments, columns and accessories, has been launched by Pye Unicam. A modular design approach enables fully integrated systems to be supplied to suit specific requirements. Both isocratic and gradient elution liquid chromatographs are available. A completely new option is a fluorimetric detector, the LC-FL, which makes possible the high sensitivity selective detection of fluorescing compounds.
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Electron beam lithography system. Varian has introduced an electron beam lithography system which has air-to-air mask and wafer handling. Designated the Varian/Extrion model EBMG-20, the machine also features a four-position elevator in a vacuum chamber, which allows post-exposure curing of electron resists. The EBMG-20 system has the ability to expose any size mask up to 6 inches square. Hardware and software available enable direct exposure of wafers up to a 5-inch diameter.
Circle No. 47 on Reader Enquiry Card.

Removable needle syringes. Hamilton. A range incorporating a removable needle has now been added to the Hamilton 700 series syringes. This enables the user to replace bent or plugged needles in the field. The needle threads securely into place and cannot be pulled off by mistake and there is virtually no dead volume.
Circle No. 48 on Reader Enquiry Card.

Wafer cooling system. Varian has introduced a fixture for its Wayflow processing station. Called Waycool, this fixture operates by controlling the wafer temperature during ion implantation, and can boost throughput by as much as 500% for high doping level applications. Beam powers up to approximately 100W can now be used without appreciable wafer heating or degradation of photoresist materials.
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APPOINTMENTS VACANT

MEDICAL RESEARCH COUNCIL
LABORATORY ANIMALS CENTRE
TWO RESEARCH OFFICERS

One post is in the Centre's Accreditation Department. Its duties will include visiting commercial breeders of laboratory animals throughout Great Britain who are accredited or recognised by the Centre.

One post is in the Director's Department and is concerned with a research programme into Specific Pathogen Free cats, with some administrative responsibility for the day-to-day running of the department.

Candidates for either post should have at least two years' postgraduate experience in biology or zoology, a knowledge of small animals would be an advantage but not essential. Salary according to age, qualifications and experience plus £354 London Weighting. Contributory Superannuation Scheme.

Applications, together with a copy of curriculum vitae and the names of two professional referees, should be addressed to. The Director, M.R.C. Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF.

MRC
Medical Research Council

9621(A)

UNIVERSITY OF NAIROBI
KENYA

Applications are invited for the post of

ASSOCIATE PROFESSOR
in the
DEPARTMENT OF CHEMISTRY

Applications are invited from candidates who have considerable experience in teaching and research as evidenced by published work. Candidates without a Ph D will not be considered. The appointee will be required to undertake academic leadership in the field of Inorganic Chemistry with regard to both teaching and research activities at undergraduate and postgraduate levels

Salary. K£3,864 to K£4,488 p.a. (K£1=£1.34 sterling) The British Government may provide salary supplementation of £5,784 p.a. (sterling) for married appointee or £3,876 p a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages Family passages, non-contributory medical scheme, S.S.S.F. or F S.S.U., housing allowance.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent to the Registrar (Recruitment and Training), University of Nairobi, P.O Box 30197, Nairobi, Kenya, by April 1, 1979

Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address.

9697(A)

Guy's Health District
GUY'S HOSPITAL

The Department of Clinical Chemistry is looking for a

BASIC GRADE BIOCHEMIST

(Salary Scale. £3,486 to £4,899 p a. depending on age and experience, plus £354 London Weighting)

To take up a research post for a period of two years in radioimmunoassay.

For application forms, please contact the Personnel Officer (Professional and Technical), on 01-407 7600 Ext 3462 Personnel Department, Guy's Hospital, St. Thomas Street, London, SE1 9RT Closing date March 2, 1979

LAMBETH, SOUTHWARK & LEWISHAM A H A (T)

9614(A)

UNIVERSITY OF LEICESTER
LECTURER
in the
DEPARTMENT OF CHEMISTRY

Applications are invited from graduates for a Lectureship in Physical Chemistry. The successful candidate will be expected to help with the teaching of the courses offered by the Department.

Salary according to qualifications and experience on the scale £3,883 to £7,754 (under review) with superannuation benefits.

Further particulars from the Registrar, to whom applications on the form provided should be sent by April 1, 1979 Further Chemical information from Professor M. C R Symons, Department of Chemistry

9617(A)

unilever research

Scientists and Research Assistants

We have vacancies for scientists and Research Assistants to join our Immunology Department. The Department is involved in the investigation of immunologic mechanisms in health and disease and the development of immunodiagnostic techniques. The programme of work covers interest in both veterinary and medical science.

We are looking for graduates and post-graduates with relevant experience to be involved in project teams.

Please write giving details of career history to:

Miss J. Kenny,
Recruitment & Career Development Manager,
Unilever Research,
Colworth Laboratory,
Unilever UK Central Resources Limited,
Colworth House,
Sharnbrook,
Bedford MK44 1LQ.

9669(A)

UNIVERSITY OF GLASGOW
DEPARTMENT OF DERMATOLOGY
CELL BIOLOGIST/BIOCHEMIST

Applications are invited for a Cell Biologist/Biochemist, required to join a small group studying the keratinisation of normal and pathological epidermis at the cellular and molecular levels. The successful candidate will be mainly involved with ultrastructural aspects of this work and some experience of the electron microscopy of tissues or proteins would be an advantage.

The position is available immediately and is supported, until December 31, 1983, by a programme grant from the Medical Research Council Initial appointment will be at a salary of £3,883 on range 1A of the salary scales for Research and Analogous Staff with superannuation benefit

Applications, enclosing curriculum vitae and the names and addresses of two referees, should be submitted to Dr D Skerrow, Department of Dermatology, Anderson College Building, 56 Dumbarton Road, Glasgow G11 6NU.

In reply please quote Ref No 4363M

9623(A)

National Heart and Chest Hospitals
BROMPTON HOSPITAL
JUNIOR MEDICAL LABORATORY SCIENTIFIC OFFICER

required for Haematology Department. Candidates should have either four 'O' levels (including mathematics and two science subjects) or two 'A' levels (including chemistry) or a degree

Application form available from Miss J. A Jenks, Personnel Manager, Brompton Hospital, Fulham Road, London SW3 6HP Tel. 01-352 8121, Ext. 4357.

9618(A)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
(University of London)
Keppel Street WC1E 7HT
DEPARTMENT OF MEDICAL MICROBIOLOGY
MEDICAL LABORATORY SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

is required to work up to two years on the viruses of African haemorrhagic fevers. Candidates must be graduates or hold H.N.C./H.N.D or an equivalent qualification and have had experience in electron microscopy Inclusive salary, depending on experience, rises to a maximum of £4,284 (Scientific Officer) and £6,123 (Senior Scientific Officer) Pensionable employment. Applications, consisting of full career and education details and naming two referees, should be sent to the Secretary (A1) at the School.

9606(A)

ATHROFA GOGLEDD-DD CYMRU

THE NORTH E WALES INSTITUTE OF HIGHER EDUCATION

POSTDOCTORAL SCIENTIST

Applications are invited for a postdoctoral research post to work on Lipid Oxidation in Milk Products Appointment will be for two years in the first instance, with salary in the range £3,910 to £4,408 per annum The work is sponsored by the Dairy Industry and will be undertaken at the North E Wales Institute, under the guidance of Dr J C Allen

Applications should be made before February 28, 1979 to The Institute Registrar, North E Wales Institute of Higher Education, Kelsterton College, Connah's Quay, Deeside, Clwýd Tel Deeside 817531, giving full details of qualifications and experience

9655(A)

VIROLOGY

Junior Faculty Position

The Institute of Clinical Virology, University of Erlangen-Nurnberg, West Germany, invites applications for a position equivalent in research responsibility and salary to an U S Assistant Professorship The main research emphasis of this department is in the molecular biology of oncogenic/pathogenic herpesviruses Some teaching obligations in German or English language Please send curriculum vitae, list of publications, letters of recommendation, and a statement of scientific interests to:

Dr B Fleckenstein, Chairman
Institut fur Klinische Virologie
Loschgestrasse 7
8520 Erlangen, West Germany

In addition, a **Postdoctoral Fellowship** is available in the department for a research programme in herpesvirus oncology Please send applications to the same address 9646(A)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY

Babraham, Cambridge CB2 4AT

A Senior Scientific Officer vacancy exists in the Neuroendocrinology Group for an electrophysiologist preferably experienced in intracellular recording techniques The successful candidate will undertake research on the control of the secretion of hypothalamic peptides Applicants should have a first class honours degree and preferably a Ph D in a relevant subject At least 4 years relevant postgraduate research or other appropriate experience is required Salary £5,154 to £6,898 per annum Non-contributory Superannuation Scheme Application form and further details may be obtained from the Secretary of the Institute, quoting Ref No N G by March 16, 1979. 9647(A)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PHYSICAL CHEMISTRY

Lensfield Road, Cambridge
Tel No 66499 Ext 383

There is a vacancy for a skilled technician to provide research facilities in electron microscopy, X-ray diffraction and other advanced techniques to the Head of Department Excellent working conditions including University pension scheme, 37½ hour week, salary range £3,670 to £4,390 (under review)

9644(A)

OVERSEAS DEVELOPMENT

KNOW-HOW: vital to developing countries

Egypt

As a result of an ODM mission to Egypt to undertake a pre-feasibility study of forage production and animal feeds a Team of three British Technical Cooperation officers are to be recruited to assist the Ministry of Agriculture, Arab Republic of Egypt, in the field of forage research and development.

Forage Agronomist

To review existing information on the agronomy of forage, fodders and other crops used for animal production in Egypt On the basis of field information to advise on an appropriate investigation programme aimed at improving production from existing species and assist in its implementation. Advise on the introduction of appropriate species of grasses, legumes, forages, fodders and other crops for trial and assist in the preliminary examination of these. In association with the Farm Systems/Farm Business Management Specialist, determine the potential value of existing and introduced species in improved farming systems and assist in their introduction and testing in such systems. Train counterpart staff in all aspects of the agronomic investigation programme. Develop proposals for a systematic programme of overseas training for counterpart staff in relevant fields of work. Applicants should have degree in agriculture or appropriate science with experience in tropical forage agronomy and the design, planning and execution of field experiments.

Appointment 2 years. Salary (UK taxable) in range £6400-£9500 pa plus variable tax free overseas allowance in range £570-£1575 pa (Ref 331X).

The post is wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, children's education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, quoting reference, stating post concerned, and giving details of age, qualifications and experience to:—

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London SW1E 5DH

9654(A)

ODM

HELPING NATIONS HELP THEMSELVES

Electrophysiologist/ Neuro Pharmacologist

The Biology Department of ICI Pharmaceuticals has consistently been at the centre of the company's research into new formulations that will improve health. Considerable effort and investment is being put into the investigation of the basic mechanisms involved in the action of centrally acting drugs, analgesics and the discovery of psychotropic agents and drugs affecting respiration.

We need now to strengthen the department by appointing an Electrophysiologist/Pharmacologist. You will be a senior member of a team initially working closely with our analgesics group as well as collaborating with multidisciplinary teams of chemists and biochemists in the other areas referred to above.

You will establish and head a laboratory concerned with research in this field, and will need to have a background which demonstrates a proven record of relevant research in one of these areas combined with a good general knowledge of relevant electrophysiological techniques. The post will be attractive to a scientist who wishes to continue in a career devoted to research.

The Division's research laboratories are situated in pleasant surroundings but within easy reach of main road and rail routes. Conditions of service, salary and assistance given to married staff in moving house are designed to attract and retain staff of high calibre.

Applications in writing should be addressed to:

Mr. M. F. Losse,
Personnel Officer,
ICI Pharmaceuticals
Division,
Mereside Research
Laboratories,
Alderley Park,
Macclesfield, Cheshire

9671(A)

Chairperson Department of Oncology

Montefiore Hospital & Medical Center

We seek an outstanding Clinician-Scientist to fill this endowed position as the head of a distinguished hospital department. The missions include clinical care, specialty training of residents and fellows, and a major commitment to clinical investigation with basic science interaction. The candidate should be eligible for professorial appointment at the Albert Einstein College of Medicine. Salary and benefits appropriate to position are provided. All interested persons should submit a curriculum vitae and bibliography to:

Laurence Finberg, M.D.,
Chairperson, Search Committee,
c/o Department of Pediatrics,
Montefiore Hospital & Medical Center
111 E. 210th St., Bronx, NY 10467, USA.

Montefiore Hospital & Medical Center is an Equal Opportunity Employer M/F.

9658(A)

MEDICAL RESEARCH COUNCIL

LABORATORY OF MOLECULAR BIOLOGY

RESEARCH ASSISTANT

required to join a group working on chemical aspects of peptide synthesis. Candidates should possess a degree or equivalent qualification in chemistry, biochemistry, or a related science subject. Initial salary on the scale £3,261 (-4680) with good prospects for promotion to research officer grade and permanency.

Applications, giving details of qualifications, experience, and names of two referees should be sent to me at this address as soon as possible.

Dr R. C. Sheppard
MRC Laboratory of Molecular Biology
The Medical School, Hills Road
Cambridge CB2 2QH.

9633(A)

UNIVERSITY OF BATH

Applications are invited for a

LECTURER IN PHYSICS

Current research areas are Solid State Physics, Ultrasonics, Underwater Acoustics. Experience in one of these areas could be an advantage but other research interests would be acceptable.

Commencing salary will be set in the lower part of the range (£3,883 to £7,754 per annum).

Further particulars and application forms are obtainable from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting reference number 79/33/N. Closing date for applications: March 16, 1979.

9631(A)

UNIVERSITY OF STRATHCLYDE

Applications are invited for a

POSTDOCTORAL RESEARCH FELLOW

or

GRADUATE RESEARCH ASSISTANT

in the

DEPARTMENT OF BIOCHEMISTRY

The work, which is financed by M.R.C., will be concerned with the structure, biosynthesis and function of renal glycoproteins. Some experience with protein or carbohydrate chemistry is desirable but not essential. The successful applicant will have opportunity to study with others working in a variety of areas of glycoprotein research and collaboration with medical colleagues will be encouraged.

The appointment will be for three years. Commencing salary £3,883 per annum on Range IA of the national salary structure for research and analogous staff. Superannuation benefit.

Written applications (quoting R6/79) and naming two referees should be forwarded to Professor R. D. Marshall, Department of Biochemistry, University of Strathclyde, The Todd Centre, 31 Taylor Street, Glasgow G4 0NR, from whom further information may be obtained.

9619(A)

QUEEN ELIZABETH COLLEGE KENSINGTON

(University of London)

LECTURER IN BIOCHEMISTRY

Applications are invited for the post of Lecturer in Biochemistry who will be required to take part in undergraduate teaching programmes up to Final Year Honours standard. The department has a strong interest in human biochemical genetics; a research interest in the field of mammalian molecular genetics would be an advantage but is not essential, and applications from people with research interests in other areas are encouraged. Salary scale £3,909 to £7,754 per annum, starting salary depending on qualifications and experience. London allowance of £450 per annum is paid in addition.

Apply to the College Secretary, Queen Elizabeth College, Campden Hill Road, London W8 7AH, from whom further particulars and an application form may be obtained. Closing date: March 23, 1979.

(Potential applicants wishing to visit the department informally are asked to make an appointment with the secretary of the Department of Biochemistry.)

9662(A)

WESTMINSTER MEDICAL SCHOOL LEUKAEMIA

RESEARCH TECHNICIAN

Applications are invited from suitable persons for the post of Research Technician in the Department of Haematology at Westminster Hospital. The research concerns human bone marrow transplantation and treatment of leukaemia. The post gives the opportunity for obtaining the F.I.M.L.S. degree by thesis.

The salary and conditions of employment are comparable to those agreed by the Whitley Council.

Applications should be sent as soon as possible to Dr A. J. Barrett, Department of Haematology, Westminster Hospital, Dean Ryle Street, London SW1.

Intending applicants may visit the laboratory by appointment with Dr Barrett, 01-828 9811, ext. 2121.

9699(A)

Organic Chemist (PhD)

Roussel Laboratories Limited is part of an international group engaged in the manufacture and marketing of ethical pharmaceutical and allied products The research group of our R & D and manufacturing complex at Swindon, Wiltshire, forms an important part of the worldwide company research effort concerned with the discovery of new therapeutic agents

We have a vacancy for an Organic Chemist in the Chemical Research Department to undertake the design and synthesis of novel compounds for biological evaluation

The vacancy requires a person with innovative skills in preparative organic chemistry combined with a sound basic knowledge of chemistry Accordingly, candidates should have a good honours degree and have obtained recently or be in the process of completing a PhD degree in Organic Chemistry

Conditions of service are excellent, including generous assistance with relocation to this attractive part of Wiltshire

Please apply on our standard application form available from Rosemary Curtis, Assistant Personnel Manager, Roussel Laboratories Limited, Kingfisher Drive, Covingham, Swindon, Wilts SN3 5BZ.
Tel Swindon (0793) 24411

ROUSSEL

9615(A)

UNIVERSITY OF BRISTOL
DEPARTMENT OF THEORETICAL CHEMISTRY
THEORETICAL STUDIES OF ELECTRON-MOLECULE COLLISIONS (DISSOCIATIVE RECOMBINATION PROCESSES)
(Re-advertisement)

Applications are invited for a Science Research Council-supported postdoctoral research assistant to work on theoretical calculations of electron-molecule collision processes of the type $e + AB^+ \longrightarrow AB^* \longrightarrow A + B$, with particular emphasis upon non-adiabetic (curve crossing) effects The project is initially funded for 21 to 23 months (depending upon initial salary), with prospect of further extension. The work will also include co-operation with Professor P G Burke's research group at Daresbury Laboratory Applicants should write as soon as possible to Dr J Gerratt, Department of Theoretical Chemistry, University of Bristol, Bristol BS8 1TS, enclosing curriculum vitae and names and addresses of two referees 9613(A)

Hampshire Area Health Authority
SOUTHAMPTON GENERAL HOSPITAL

Applications are invited for the post of Basic Grade Biochemist in the University Department of Chemical Pathology and Human Metabolism The post offers good opportunities for development and research work in trace metal analysis, and is initially for a period of three years The position would best suit a graduate with a strong chemistry background Whitley Council conditions of service apply. Salary scale £2,991 to £4,899 depending on qualifications and experience

Job description, further particulars and application forms are available from Personnel Department, Tremona Court, 30-32 Tremona Road, Southampton. Telephone Southampton 777222 Ext 3556 Arrangements to visit the department may be made with Mrs B Loyd, Southampton General Hospital Telephone Southampton 777222 Ext. 3922 Closing date February 28, 1979 9653(A)

Agriculture & Horticulture
LIFE SCIENTIST?
Become a Specialist Adviser

The Science Service of the Agricultural Development and Advisory Service provides farmers and growers with a wide range of vital technical information and advice which is supported by investigation and development work carried out from 12 regional centres across the country

Opportunities currently exist for graduates to become Specialist advisers in the following science disciplines Full training will be given where necessary

MICROBIOLOGY

Study of micro-organisms of importance in the production, processing, preservation and storage of farm and horticultural products, treatment and disposal of waste products and effluents; bacterial pathogens causing plant disease, hygiene of dairy products and quality control of farm water supplies *Ref. S(H)906*

ENTOMOLOGY

Primarily, the diagnosis of causes of pest damage to crops, identification of these pests, assessment of their importance and significance, and recommendations for prevention and control *Ref. S(M)906*

PLANT PATHOLOGY

Diagnosis of plant diseases caused by fungi, bacteria and viruses Special interest in the assessment of the damage done and losses caused by plant diseases, and in seeking preventive or control measures *Ref. S(K)906.*

NUTRITION CHEMISTRY

Nutrition of farm animals, the nature and composition of feeding stuffs, formulation of rations for livestock, and biochemical aspects of the cultivation, processing, preservation and use of animal feeds *Ref· S(J)906*

SOIL SCIENCE

Primarily, all aspects of soils—physical, chemical and biological—that affect the nutrition, growth and production of agricultural and horticultural crops This involves both field advisory work and investigations to study conditions of optimum production and the reasons for partial or complete failures in crop production *Ref S(L)906*

Candidates, normally aged under 28, should have a good honours Degree or equivalent in an appropriate dicipline *Final year students may apply*

Salary, starting between £2,995 and £3,810, according to age, qualifications and experience, rises to £3,920 Promotion prospects Non-contributory pension scheme

Write for full details and application form (to be returned by 16 March 1979) to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours) *Please quote appropriate reference*

Men and women may apply 9688(A)

Agricultural Development and Advisory Service.

Ministry of Agriculture, Fisheries and Food

Head of Biochemical Research

As a result of the Company's policy of offering challenging careers to its scientists in every sector of its business, a senior position will shortly become vacant within the group's Central Research Laboratory at Luton. This is a crucial and demanding job as it covers the whole of the Company's analytical and biochemical research, and the application of this work to the production principally of beer, but also of spirits, wines and soft drinks.

Further responsibilities and development of the career of the chosen person will depend as much on the individual as on the Company's expanding activities.

We are therefore looking for a well qualified, young scientist who has already demonstrated an aptitude for research, an inventive mind and the ability to apply it where it matters most – in production.

Remuneration will reflect both the seniority of the position and the experience of the successful applicant.

Applications should be sent to:
Miss P. M. Donnelly, Personnel Officer,
Whitbread & Co. Limited, The Brewery,
Chiswell Street, London EC1Y 4SD.
Telephone: 01-606 4455.

9679(A)

WHITBREAD

AGRICULTURAL RESEARCH COUNCIL

Poultry Research Centre
West Mains Road
EDINBURGH EH9 3JS

Applications are invited for the following vacancies:

CHEMIST/BIOCHEMIST/NUTRITIONIST

A Chemist/Biochemist with relevant nutritional experience is required by the Nutrition Department at the above Centre. The appointee will work on the evaluation of feedstuffs as energy sources for poultry. The primary objective will be to understand digestive and metabolic processes which influence and limit energy values of feedstuffs and the potential for modifying these limits. Definition of energy values and the development of experimental methodology will also be involved. Close liaison with scientists working on energy metabolism would be envisaged. (Ref. No. 796.)

Nutritionist or Biochemist with a special interest in whole animal metabolism and simulation modelling is required by the Nutrition Department at the above Centre. The appointee will work on the development of simulation models of biological processes which are relevant to poultry nutrition and production. Initially, models of metabolism in the growing animal will have the highest priority. Research work on the biochemical/metabolic level will support model development. Candidates may have a variety of experience or interests but must demonstrate an ability to relate "whole animal" metabolic and biochemical concepts to practical nutritional decisions and must have a genuine interest, and preferably some experience, in the use of computer modelling techniques in biology. (Ref. No. 797).

Qualifications: 1st or upper 2nd class honours degree and preferably a Ph.D.

Salary: In the scale £4,101 to £5,448 at H.S.O. level and £5,154 to £6,898 at S.S.O. entry. Entry at the higher level requires at least 4 years postgraduate experience. Non-contributory superannuation scheme. 22 days annual leave, plus 10½ public/privilege holidays.

Application forms can be obtained from the Secretary at the above address. These should be completed and returned to him not later than Friday, March 16, 1979, quoting our reference number.

9692(A)

PUBLIC HEALTH LABORATORY SERVICE BOARD

P.H.L.S. Centre for Applied Microbiology and Research
Porton Down

Applications are invited from experienced microbiologists for two senior posts in the rapidly expanding field of microbiological laboratory safety.

1. A **Senior Grade Microbiologist** to act as the Deputy Safety Officer for the Centre and to be responsible for the monitoring of equipment, facilities and experimental procedures within the Centre. Additionally, he/she will be largely responsible for devising and implementing training programmes in microbiological laboratory safety.
2. A **Senior Grade Microbiologist/Chief Medical Laboratory Scientific Officer** is required to undertake research into and development of methods of assessing the safety of equipment, facilities and experimental procedures, including the stability of airborne microorganisms. He/she will be expected to contribute to the training programmes in microbiological laboratory safety, offered by the Centre.

In addition to demonstrated abilities as microbiologists, the successful candidates will be expected to have a good knowledge of safety legislation and codes of practice relevant to microbiological laboratory procedures.

Some experience in the monitoring and use of radio-isotopes would be an advantage. There may be a requirement for the successful candidates to travel to hospitals and other laboratories to participate in tests of equipment or facilities on site.

Salary scales: Senior Grade Microbiologist, £5,451 to £6,837; Chief Medical Laboratory Scientific Officer, £5,472 to £6,192.

The appointments will be under P.H.L.S. (N.H.S.) terms and conditions of service.

Further details of the posts may be obtained from Dr R. A. Bassett, Public Health Laboratory Service, 61 Colindale Avenue, London NW9 5EQ, to whom applications with full career details and the names and addresses of three referees, should be sent by March 15, 1979. 9694(A)

UNIVERSITY OF EDINBURGH

Faculty of Medicine
DEPARTMENT OF BIOCHEMISTRY

UNIVERSITY DEMONSTRATOR

Applications are invited for the post of University Demonstrator which is tenable from October 1, 1979, or earlier by arrangement.

The initial salary will be at a suitable point on the scale £3,384 to £4,882 p.a.

The major duties of the University Demonstrator will be to participate in teaching medical, dental or science students and to engage in independent or collaborative research.

Further particulars may be obtained from the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, with whom applications (six copies), including the names of two referees, should be lodged not later than March 31, 1979.

Please quote: Reference 1017.

9691(A)

THE UNIVERSITY OF HULL

DEPARTMENT OF ZOOLOGY

Applications are invited for a Lectureship in the Department of Zoology from October 1, 1979. The Lectureship has been established to complement the team of existing members of staff with expertise in vertebrate physiology who collectively will provide a course in Human Physiology for the newly instituted Honours School in Zoology. The successful candidate will have a good honours degree in Zoology, Biochemistry or Physiology and/or be medically qualified in the general area of physiology and have demonstrated a capacity for independent research in a major area of physiology.

Salary for non-medically-qualified candidates within the scale £3,883 to £7,754 per annum (under review) plus U.S.S. benefits.

Applications (six copies) giving details of age, qualifications and experience, together with the names of three referees should be sent by March 8, 979 to the Registrar, The University of Hull, Hull, HU6 7RX from whom further particulars may be obtained.

9601(A)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF CHILD HEALTH

Applications are invited for the post of LECTURER in DEVELOPMENTAL BIOLOGY in the Department of Child Health. The Department (Professor David Hull) was established in September 1972.

The succesful applicant should have experience of research into the biology of the foetus and the newborn in both animals and man and to be familiar with both physiological and biochemical methodology. He/She should also have had some experience of undergraduate and postgraduate teaching.

Salary will be on the non-clinical lecturer scale with membership of U.S.S. The starting salary will depend on qualifications, age and experience. The successful candidate will be expected to assume duty as soon as possible.

Other particulars and a form of application may be obtained from the Deputy Registrar, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, to whom completed applications should be returned by the March 1, 1979. 9599(A)

THE OPEN
UNIVERSITY
FACULTY
OF SCIENCE

Lectureship in Biology

Applications are invited for a tenured lectureship in Vertebrate Physiology. The person appointed will be involved in the preparation and maintenance of the Biology Discipline's 2nd and 3rd level courses in Physiology, and will be expected to take an active interest in the development of the discipline's future offerings in the field of continuing education He/she will be expected to undertake research either independently or in association with one of the existing research groups in the discipline The successful candidate should have a good research record and teaching experience in a physiological area

Commencing salary will be within the lecturer scale £3,883 to £7,754 p a with U S S benefits

Application forms and further particulars are available from The Recruitment Office (JD3661/2), The Open University, P O Box 76, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63404 there is a 24 hour answering service on 63868 Closing date for applications 1st March 9609(A)

UNIVERSITY OF EXETER
DEPARTMENT OF PHYSICS

Applications are invited for a post of Lecturer in Physics tenable from October 1, 1979 The research field of interest is Medical Physics Preference will be given to applicants with experience in hospital departments

Commencing Salary within the range £3,883 to £5,604 p.a (under review), on the salary scale £3,883 to £7,754 p a (under review) Further particulars may be obtained from Miss Doreen Watson, University of Exeter, Northcote House, Exeter, EX4 4QJ to whom applications (8 copies) should be sent by March 2, 1979 Please quote reference No 3199 9598(A)

UNIVERSITY OF THE WEST INDIES JAMAICA

Applications are invited for the post of

LECTURER/ ASSISTANT LECTURER

in the

DEPARTMENT OF CHEMISTRY

Applicants with interest in one or more of the following Photochemistry, Electrochemistry, Radiochemistry or Gas Phase Kinetics would be preferred and an interest in energy studies would be an added advantage. The appointment is for three years to September 30, 1982.

Salary scale Lecturer, J$8,913 to J$13,917 p a, Assistant Lecturer, J$7,236 to J$7,926 p.a. (£1 sterling= J$3 38). F.S S.U. Study and Travel Grant Unfurnished accommodation will be let by the University at a rental of 10% of salary, or a housing allowance of 20% of salary will be paid Family passages.

Detailed applications (2 copies) with curriculum vitae and naming three referees should be sent direct as soon as possible to the Registrar, University of the West Indies, Mona, Kingston, Jamaica

Applicants resident in the UK should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address 9696(A)

Human Pharmacokinetics (Study Co-ordinator)

May & Baker Ltd. is an international Company, manufacturing and marketing a wide range of research based pharmaceutical products.

A vacancy has arisen for a Study Co-ordinator, reporting to the Head of the Pharmacokinetic Section of the Drug Metabolism Department, at Dagenham, Essex. The work involves the setting up, programming and monitoring of pharmacokinetic studies in healthy human volunteers and patients, in such a way that the studies are conducted according to the highest international standards. When available, the successful applicant will assist in the analysis of biologial samples and in the pharmacokinetic-analysis of results obtained.

The studies will be conducted both "in-house" and in collaboration with the Clinical Pharmacology units of several hospitals.

The successful applicant will be scientifically trained in an appropriate discipline, with good organisational ability and capable of working independently. He/she should be a graduate or an experienced technician with, ideally, a current driving licence.

Please telephone 01-592 3060 extension 2506 for an application form or write with full details of age, experience, qualifications and present salary to

M&B May&Baker

Mrs. P. M. Clark, Personnel Officer, (Scientific Staff), May & Baker Ltd., Dagenham, Essex RM10 7XS, quoting reference number 12/N/1 9663(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the post of

PROFESSOR IN BOTANY

Applicants should possess a Ph D or equivalent in Botany with at least ten years of teaching at University level. Applicants must also have had considerable research experience as evidenced by published work in any of the important areas of plant sciences They should also have a capacity for exercising leadership in undergraduate and postgraduate work as well as considerable administrative experience.

Salary scale. K£4,632 to K£5,562 p a (K£1=£1 34 sterling) The British Government may supplement salary by £6,552 p a. (sterling) for married appointee or £4,644 p a (sterling) for single appointee (reviewed annually and normally free of tax) and provide children's education allowances and holiday visit passages Family passages. superannuation scheme, medical aid scheme, various allowances

Detailed application (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar, University of Nairobi, P O. Box 30197, Nairobi, Kenya, by April 5, 1979

Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address 9695(A)

COLEG PRIFYSGOL CYMRU THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH

LASER STUDIES IN CHEMICAL PHYSICS

Following the award of a major grant from the Science Research Council to promote the application of new laser systems to spectroscopy, we wish to invite applications for the following appointments (from 1 April, 1979, or as soon possible there-after);

1. TWO POST-DOCTORAL RESEARCH ASSOCIATES, Grade 1A, starting salary in the range £3,883 to £4,382 depending on age and experience; (2 year appointments).

2. ONE POST-GRADUATE RESEARCH ASSISTANT, Grade 1B, starting salary in the range £3,384 to £3,883. (3 year appointment). Holders of this position may be eligible to submit for a higher degree of the University of Wales.

The persons appointed will be expected to work in a laser group encompassing the research interests of Professor W. J. Jones, Dr. P. A. Freedman, Dr. J. O. Williams and Professor G Williams. A wide range of lasers and spectroscopic instrumentation is available to promote studies in (a) time-resolved photochemistry and photophysics utilising a synchronously-pumped mode-locked/cavity-dumped argon ion-dye laser system (P.G.R.A and one P.D.R.A.) and (b) the spectroscopy of gaseous molecules cooled to near 0K in a supersonic jet (one P.D.R.A.).

Application forms and further particulars may be obtained from The Registrar, The University College of Wales, Old College, King Street, Aberystwyth SY23 2AX to whom completed application forms should be submitted as soon as possible. 9642(A)

Senior Scientific Officer/ Higher Scientific Officer/ Scientific Officer

£2,839 to £6,898

Applications are invited for a pensionable post in the Plant Pathology Division of the Department of Agriculture situated at Newforge Lane, Belfast.

The successful candidate will be expected to take part in research on crop disease problems (primarily horticultural) and to assist in the provision of a specialist advisory service. The person appointed may also be required to undertake teaching duties in the Faculty of Agriculture and Food Science, Queens University, Belfast.

Senior Scientific Officer

Over 25 and under 32 years of age on December 31, 1979, with a 1st or 2nd class honours degree in Botany or Microbiology and at least 4 years' relevant postgraduate experience.

Higher Scientific Officer

Under 30 years of age on December 31, 1979, with an honours degree as above and at least 2 years' appropriate postgraduate experience.

Scientific Officer

Under 27 years of age on December 31, 1979, with an honours degree as above.

Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

The Civil Service Commissioners may decide to interview only those applicants who appear from the information available (including the level of academic attainment and relevant experience) to be best qualified.

Salary scales

Senior Scientific Officer	£5,154 to £6,898
Higher Scientific Officer	£4,101 to £5,448
Scientific Officer	£2,839 to £4,415

(The salary scales will be subject to review from April 1, 1979.)

Grading and starting salary will be related to qualifications and experience.

There are prospects of promotion to Principal Scientific Officer, £6 609 to £8,461.

Please write or telephone for an application form quoting job reference SB 105/79/NM to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than March 2, 1979. 9656(A)

NORTHERN IRELAND
CIVIL SERVICE

UNIVERSITY OF EDINBURGH
LECTURER IN SEED TECHNOLOGY

The Edinburgh School of Agriculture invites applications for a University Lectureship with responsibility for the M.Sc./Diploma Course in Seed Technology. This [illegible] month postgraduate course, which is supported by ODM, has emphasis on technical management for seed production, particularly overseas. Applicants should be University graduates with experience of seed production overseas. This is a three year appointment, in the first instance, commencing at the latest from October 1979 on the Lecturer scale £3,883 to £7,754 per annum.

Further particulars can be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL. Applications should arrive, if possible, not later than 17 April 1979. Please quote Reference 1013.

9629(A)

KING'S COLLEGE HOSPITAL

DEPARTMENT OF HAEMATOLOGY

A Graduate Research Assistant to participate in a three year project investigating the immunological and cytochemical properties of lymphoid cells in malignant disorders. Training will be given in the necessary techniques and it is possible that the project will provide the basis for a higher degree.

The appointment will be tenable from April 1, 1979 for one year in the first instance, renewable annually.

For further details, contact Dr M. V. Joyner on 01-274 6222 ext. 2735. Application form and job description available from the District Personnel Department, King's College Hospital, Denmark Hill, London SE5 9RS. Tel: 01-274 6222 ext. 2254.

Closing date: March 2, 1979.

9676(A)

BIOCHEMICAL SOCIETY

Applications are invited for a senior post in the Society's Editorial Office. The post involves responsibility for editorial processing of material for the **Biochemical Journal** and other Biochemical Society publications, and the successful applicant will be required to act as a deputy for the Editorial Manager. As well as a degree in biochemistry or related subject it is essential that candidates (probably of age 35-45) should have had sound postgraduate experience of scientific editorial work and academic publishing and be capable of supervising the work of others. A salary commensurate with the seniority of this post will be offered. Please apply in writing, giving the names of two referees, to the Eexcutive Secretary, Biochemical Society, 7 Warwick Court, London WC1R 5DP.

9622(A)

SENIOR HISTOPATHOLOGIST
THE ST GEORGE HOSPITAL
with conjoint appointment as Clinical Professor or Clinical Associate Professor
School of Pathology
University of New South Wales Australia

Suitably qualified medical practitioners are invited to apply for appointment as Senior Histopathologist at this 550 bed teaching hospital of the University of New South Wales. The appointee may also be offered the title of Clinical Professor (conjoint appointment) or Clinical Associate Professor (conjoint appointment) and as such would be responsible to the University of New South Wales for teaching in the School of Pathology, with access to the facilities of that school.

The Histopathology Division provides a comprehensive service to the Hospital and is staffed with a second full-time specialist and two Registrars. A full-time Senior Lecturer in Pathology is also on site.

Salary is at the Senior Staff Specialist rate, currently $A39,364 p.a. Other conditions in terms of the Medical Officers' Hospital Specialists' (State) Award include study leave of three months for each five years' service and limited right of private practice in terms of the procedures laid down by the Health Commission of New South Wales.

Written applications, including the names and addresses of three referees close with the Chief Executive Officer, The St George Hospital, Kogarah, 2217, on April 12, 1979. Enquiries concerning hospital duties should be directed to the Medical Superintendent, The St George Hospial, phone (02) 588 1111, and on Uiversity matters to Professor A. Lykke (02) 662 2770.

9537(A)

CHILDREN'S HOSPITAL BIRMINGHAM
TECHNICIAN OR BIOLOGICAL SCIENCE GRADUATE

required for a research post. The successful applicant will be concerned with a leukaemia and non-Hodgkins Lymphoma study. Previous experience in immunology helpful but not essential. Appointment for one year in the first instance.

Salary according to experience and qualifications.

For further details and an application form please contact Dr F. G. H. Hill, Consultant Haematologist, Haematology Department, The Children's Hospital, Ladywood Middleway, Birmingham B16 8ET. Telephone: 021-454 4851. Closing date: March 15, 1979. 9590(A)

PROFESSOR OF BIOCHEMICAL PHARMACOLOGY AND CHAIRPERSON
DEPARTMENT OF PHYSIOLOGY BIOCHEMISTRY AND PHARMACOLOGY

The New York State College Veterinary Medicine at Cornell Un versity invites nominations and applica tions for the position of Chairperso of the Department of Physiology, Bi chemistry and Pharmacology. Th speciality of the applicant should within the discipine of biochemica pharmacology. Credentials shoul include evidence of outstandin research achievements and potentia administrative ability and considerab teaching experience, and there shoul be a demonstrated commitment to e cellence in teaching and research.

The department is multidisciplinar with responsibility for teaching, r search and service. The 17,000 squar feet of teaching and research labor tory space and 6,000 square feet specialised animal-holding space a well-equipped for research and instru tion on a wide range of subjects an species. The department is also engage in interdisciplinary research projec and training programmes in toxicolog and comparative gastroenterology.

Applicants should submit thei curriculum vitae, and request that thre letters of recommendation be sen directly to the Search Committe Applications accepted through June 1979.

Please address all correspondence t

Professor R. H. Wasserman,
Chairman
Search Committee
Department of Physical Biology
New York State College of
Veterinary Medicine
Cornell University, Ithaca,
New York 14853.

Affirmative Action/Equal Opportunit
Employer

9670(A)

UNIVERSITY OF LEICESTER
PHYSICS RESEARCH Ph.D.

The Department of Physics offer excellent research opportunities leading to the degree of Doctor of Philosophy in the following areas:

IONOSPHERIC PHYSICS
NUCLEAR ELECTRONICS
QUANTUM MOLECULAR PHYSICS
SOFT X-RAY SPECTROSCOP
SOLID STATE PHYSICS
THEORETICAL PHYSICS
X-RAY ASTRONOMY

Further details and application form may be obtained from Professor J.L. Beeby, Department of Physics, University of Leicester, Leicester LE1 7RH

9488(A)

UNIVERSITY OF CALIFORNIA LOS ANGELES
POSTDOCTORAL SCHOLAR AND ADJUNCT ASSISTANT PROFESSOR

Individuals with expertise in biochemistry, lipid or protein chemistr to investigate transmembrane processe or membrane structure/function. Postdoctoral position available immediately $12,000 per year. Adjunct positio available June-September 1979 for 1- years maximum, approximate salar $15,000/9 months plus 1-2 month summer salary, requires some teachin in undergraduate course in microbiology. Send inquiries or statemen of interests, résumé and 3 letters o recommendation to Dr B. J. Wisnieski Department of Microbiology and th Molecular Biology Institute, University of California, Los Angeles California, U.S.A. 90024. An equa opportunity employer. 9677(A)

THE OPEN
UNIVERSITY
FACULTY
OF SCIENCE

Research Assistant in Physics

The post is available immediately, starting date negotiable. The successful candidate will be required to develop electronic circuitry including interfaces for various data acquisition systems used in experiments in the Physics Discipline, which include work on biomagnetism, solar flux, semiconductor physics and nanosecond spectroscopy.
Applicants should hold a good honours degree in Physics Electronics or Electrical Engineering and be competent in modern electronics.
This post is available for three years in the first instance, with the possibility of renewal for a further two years.
Commencing salary will be within the Research Assistant 1B scale £3,384 to £4,882 p.a. with U.S.S. benefits.
Application forms and further particulars are available from The Recruitment Office (JD3773/3), The Open University P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL, or telephone Milton Keynes 63404: there is a 24 hour answering service on 63868.
Closing date for applications: 1st March. 9610(A)

ST. GEORGE'S HOSPITAL
MEDICAL SCHOOL
(University of London)

POSTDOCTORAL RESEARCH ASSISTANT

urgently required for a Medical Research Council three-year project in the Department of Structural Biology to work on fine structure and histochemistry of fracture healing in long bones. Candidates should have qualifications in Biology or Zoology and be competent in all aspects of preparative techniques for, and the use of, a transmission electron microscope.

Salary in the range £3,883, £4,133, £4,382 plus London allowance £450 per annum.

Application forms and further particulars available from the Establishment Officer, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.

9683(A)

ROTHAMSTED
EXPERIMENTAL STATION
Harpenden, Herts, AL5 2JQ

SCIENTIFIC OFFICER

required to assist in a research programme concerned with virus diseases of grasses and legumes. Responsibilities will include laboratory, glasshouse and field work on virus diseases of ryegrass, clovers and field beans.

Qualifications: Pass degree, HNC or equivalent in biological sciences related to crop production. Experience in handling viruses and/or virus vectors and in field experimentation desirable but not essential. Driving licence.

Appointment in grade of Scientific Officer, £2,839 to £4,415 per annum. Starting salary according to qualifications and experience. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees and quoting Ref. 387 by March 15, 1979. Further details on request. 9687(A)

Wellcome

Chemist/Biochemist

Veterinary Research

Applications are invited from young postgraduates, probably aged 25-35, to work as a Scientist within a multi-disciplinary team, studying the ways in which penetration, tissue distribution, and metabolism are involved in resistance to insecticides.

Candidates should possess an honours degree in either Chemistry and/or Biochemistry and have experience in the use of modern analytical techniques, especially with regard to the estimations of compounds at very low levels.

The Laboratories are part of The Wellcome Group Research and Development and are concerned with veterinary pharmaceuticals and control of insect pests. They are situate on the outskirts of Berkhamsted. In addition to an attractive salary, the Group offers excellent conditions of employment including assistance with relocation expenses where appropriate.

If you are interested, please write or phone for an application form, quoting reference NA.2, to: A. J. Morris, Site Personnel Officer, The Wellcome Foundation Limited, Ravens Lane, Berkhamsted, Herts. Tel: Berkhamsted 3333, ext. 363.

1976

9661(A)

UNIVERSITY OF ZAMBIA

Applications are invited for the following posts in the DEPARTMENT OF AGRICULTURAL ECONOMICS

PROFESSOR/ASSOCIATE PROFESSOR/SENIOR LECTURER/LECTURER

Applicants should have a Ph.D., preferably with emphasis on Agricultural Economics. Experience in an African or other developing country preferred. Duties include conducting lectures and tutorials in Agricultural Economics, Marketing and Agricultural Policy for undergraduate students, plus initiating research or continuing current research into agricultural performances and policy in Zambia.

Salary scales: Professor K8,616 to K9,135 per annum, Associate Professor K8,232 to K8,616 per annum; Senior Lecturer K7,603 to K8,016 per annum; Lecturer K4,956 to K7,608 per annum (£1 sterling=K1.56). The British Government may supplement salaries in range £2,922 to £5,952 per annum (sterling) for married appointees and £930 to £3,366 per annum (sterling) for single appointees (reviewed annually and free of tax) and provide childrens education allowances and holiday visit passages. Family passages; baggage allowance; medical aid scheme; regular overseas leave. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia by March 31, 1979. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9638(A)

BUSH BOAKE ALLEN LIMITED
a division of
ALBRIGHT AND WILSON LIMITED
have a vacancy in the Process Technology Department
of the Flavour Laboratories at Walthamstow
London E17 for a

SENIOR PROCESS TECHNOLOGIST

The principle duties of the successful candidate will be to follow lines of research in connection with onion flavours. In particular he/she will study the relationship between varieties and stages of maturity of onions with their yields of flavour precursors.

Applications are invited from candidates aged 24+ with a good academic knowledge probably at post-graduate level. Experience with natural products and their chemistry and handling is essential. A knowledge of, or experience with H.P.L.C. is desirable.

An attractive salary will be offered in addition to a range of employee benefits which include a non-contributory pension scheme.

Applications in writing to:

R. J. Saunders
Recruitment and Training Manager
BUSH BOAKE ALLEN LTD.
Blackhorse Lane
Walthamstow E17
Tel: 01-531 4211

stating age, qualifications and experience.

9689(A)

Institute for Marine Environmental Research

SCIENTIFIC OFFICER
HIGHER SCIENTIFIC OFFICER
SENIOR SCIENTIFIC OFFICER

The staff of the Institute comprises multi-disciplinary teams for research on ecology of open seas and estuaries, and effects of stress and pollution Investigations in the field and by laboratory experiments include analyses of variability in natural populations and climate, quantification of ecosystem processes, formulation of mathematical simulation models and derivation of indices of environmental quality. There are four vacancies at Plymouth:

Post 293—Microbiology: for research on heterotrophic and autotrophic processes in open seas and estuaries in relation to ecosystem dynamics, organic substrates, and nutrient herbivore and detritivore cycles. Candidates preferably with post-graduate experience in microbiology and related organic chemistry.

Post 305—Statistics: to provide a consultative service, participate in statistical design, analyses and interpretation, and to develop methods for marine ecological research. Candidates preferably with experience in application of probability statistics to research in natural sciences.

Post 328—Computing: for management and development of software for research in marine ecology; also operation of two PDP-11s (RSTS/E and RSX-11M); supervision of one assistant and two data processors Candidates preferably with experience of software development for research, knowledge of FORTRAN and BASIC

Post 330—Mathematical modelling: to participate in multidisciplinary research teams, to develop deterministic and stochastic models of marine ecosystems. Candidates preferably with post-graduate experience in mathematical ecology

Academic Qualifications: For Posts 293, 305 and 330, a good Honours degree in an appropriate subject. For Post 328 a degree, HNC or HND in computing science plus relevant experience Ability to work at sea in research vessels and small boats is essential for Post 293 and would be an advantage for the other posts.

At least two years' relevant postgraduate experience is required for appointment as Higher Scientific Officer. Candidates with less or more experience may be appointed as Scientific Officer or Senior Scientific Officer, respectively.

Salary: SO—£2,839 to £4,415; HSO—£4,101 to £5,448; SSO—£5,154 to £6,898. Starting salary may be above the minimum

Staff of the Council are not Civil Servants but pay and conditions are similar to those of the Civil Service, including a non-contributory pension scheme.

For further particulars and application forms, please write, quoting the Post Number, to The Director, Institute for Marine Environmental Research, Prospect Place, The Hoe, Plymouth PL1 3DH.

Natural Environment Research Council

9607(A)

SOMERSET LEVELS PROJECT
RESEARCH ASSISTANT

required for palaeobotanical investigations of prehistoric environments in the Levels. Position tenable in the Department of Archaeology, University of Cambridge.

Salary approximately £3,000 per year; starting date, April 1, or negotiable.

Further details from:

Dr J. Coles
Department of Archaeology
Downing Street, Cambridge

Closing date: March 5.

9693(A)

UNIVERSITY OF DURHAM
DEPARTMENT OF ZOOLOGY

Applications are invited for the post of

LECTURER IN ANIMAL BEHAVIOUR

tenable from October 1, 1979 Candidates should have field experience and be able to assist with ecological teaching and with the development of Joint Honours courses with Psychology and Anthropology

Salary on the scale £3,883 to £7,754 per annum (under review) plus U S.S. Applications (3 copies) naming three referees, should be sent by February 23, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained.

9463(A)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post of

RESEARCH ASSOCIATE

to participate in research with Dr S. J Ferguson on a biochemical investigation of the Bioenergetics of Paracoccus denitrificans

Starting salary will be in the range £3,384 to £3,883 per annum (plus U S.S.).

The post, which is supported by the S.R.C , is to be filled as soon as possible, and is available for a maximum of three years

Applications (2 copies) including names of 2 referees to Miss L R. Heyes, Senate Registry, University of Birmingham, P.O Box 363, Birmingham B15 2TT, from whom further particulars may be obtained

Closing date. March 9, 1979.

9682(A)

UNIVERSITY OF LEICESTER
DEPARTMENT OF PHYSICS

RESEARCH DEMONSTRATOR

Applications are invited for a post of Research Demonstrator in Condensed Matter Physics. He/she will be required to undertake, under the direction of the Head of Department, tutorial or demonstrating work within the Department up to a maximum of twelve hours a week, he is not expected to give lecture courses The successful applicant will be required to work for the degree of Doctor of Philosophy with the condensed matter physics group. The interests of the group include the study of disordered matter and of metal surfaces

Salary within the range £3,215 to £3,384 to £3,634.

Further particulars from the Registrar to whom applications on the form provided should be sent by April 1, 1979

9678(A)

MEDICAL RESEARCH COUNCIL
BIOCHEMICAL PARASITOLOGY UNIT
MOLTENO INSTITUTE
UNIVERSITY OF CAMBRIDGE
RESEARCH TECHNICIAN

to assist a group studying the biochemistry of parasitic protozoa The work involves operation and basic maintenance of an analytical ultracentrifuge. Microbiological/Biochemical training and experience in optical/photographic systems essential HND or equivalent required.

Salary range £3,190 to £3,838 (under review)

Applications to The Director, Medical Research Council Biochemical Parasitology Unit, The Molteno Institute, University of Cambridge, Downing Street, Cambridge CB2 3EE.

9686(A)

QUEENSLAND INSTITUTE OF TECHNOLOGY
Brisbane—Australia
HEAD OF SCHOOL OF APPLIED SCIENCE

The Institute is seeking a suitable person to fill this senior post Salary is $A33,061 per annum. Full details are available from the Agent-General for Queensland, 392 The Strand, London WC2R 0LZ or telephone (01) 836 3224

Written applications quoting V. 22/79 together with telephone contacts, full details and the names of three referees should reach the Personnel Officer, Q I.T , George Street, Brisbane 4000 Australia by May 18, 1979

9650(A)

THE ROWETT RESEARC
INSTITUTE
Bucksburn, Aberdeen AB2 9SB
APPLIED NUTRITION DEPARTMENT
RESEARCH OFFICERS
(2 posts)

The Institute invites applicati
from suitably qualified candidates
two research posts within its Appl
Nutrition Department

1. The successful applicant for
first post would be expected
work on aspects of the nutriti
of cattle, paying particular att
tion to optimalising the use
resources in the production
meat.
This post will be in the Sen
Scientific Officer (£5,154 to £6,
per annum) or Principal Scient
Officer (£6,609 to £8,461
annum) grades and starting sal
depends on experience. At le
four years' postgraduate expe
ence is required in relevant fie
of research.
2. The second post is for a per
who would be expected to stu
the nutrition of the very you
pig with particular reference
amino acid requirements
their relationships to the dieta
energy supply.
This appointment will be
Scientific Officer (£3,294 to £4,
per annum) or Higher Scienti
Officer (£4,101 to £5,448
annum) grades and starting sal
depends on experience At le
two years' relevant postgradu
experience is required for H.S
grading.

Qualifications required: First
upper second class honours degree
one of the biological sciences or
agriculture, and an interest in
practical application of science in
animal industry.

There is a non-contributory pensi
scheme.

Further details of the post may
obtained from the Secretary of
Institute, to whom applications shou
be submitted together with the nam
of two people to whom reference c
be made Closing date March 16, 19

9643(A

UNIVERSITY OF GLASGO
DEPUTY RADIATION PROTECTION OFFICER

Applications are invited for
above appointment in the Univers
Radiation Protection Service. Ap
cants, preferably with a degree
physics, should have several years'
perience in operational health phys
or in handling radioisotopes and
capable of dealing with all aspects
radiological protection in the Univ
sity

The successful applicant, under
overall supervision of the Radiati
Protection Officer, will be responsil
for the day-to-day operation of
University Radiation Protection S
vice. Duties will include advising
the safe use of radioisotopes, X-r
machines, lasers and U.V. sourc
conducting routine surveys of rad
tion areas, assisting with the dispo
of radioactive waste, coping with ra
ation emergencies, maintaining hea
physics records, and supervisi
ancillary staff.

To maintain the good worki
relationship which exists between
Service and the departments it serv
preference will be given to candida
of suitable personality with an abil
to liaise and communicate with work
in other disciplines

Salary will be at an appropri
point on Grade 1A (£3,883 to £6,5
interim) of the salary scales for Ot
Related Staff.

Further particulars may be obtai
from the Secretary of the Univers
Court, (Room 18), University
Glasgow, Glasgow G12 8QQ, w
whom applications (8 copies), toget
with the names and addresses of t
referees, should be lodged not la
than March 8, 1979

In reply please quote Ref
4374M 9652(A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
SENIOR LABORATORY SCIENTIFIC OFFICER/ LABORATORY SCIENTIFIC OFFICER

Required in the Department of Biochemistry to take charge of the Undergraduate Teaching Laboratory. The appointee will be expected to be totally responsible for the preparation work for Pre-Clinical Medical and Dental Classes during term-time. A part-time appointment might be possible.

Full-time salary, according to experience, in the range:—

Laboratory Scientific Officer: £3,261 to £4,680 plus £354 London Weighting p.a.

Senior Laboratory Scientific Officer: £4,347 to £5,769 plus £354 London Weighting p.a., together with superannuation.

Enquiries to Dr. C. W. Parr, Tel. 01-247 0644 Ext. 20. Applications to the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, quoting Ref. B/1/79.

9603(A)

UNIVERSITY OF LONDON
READERSHIP IN PHYSIOLOGY AT QUEEN ELIZABETH COLLEGE

The Senate invites applications for the above Readership to be held at the Department of Physiology (Head of Department: Professor D. L. Yudilevitch, M.D.). The Reader will be an experienced physiologist of proven research ability. The Department has been expanding its research activities in the past few years and is now well-equipped for studies in its current interests. Queen Elizabeth College is located in Kensington in a pleasant neighbourhood near Holland Park.

The successful candidate will be expected to develop his/her own research, supervise postgraduate research students and to contribute to undergraduate teaching.

Applications (10 copies) must be received not later than March 30, 1979, by the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, from whom further particulars (including the salary scale) should first be obtained.

9684(A)

UNIVERSITY OF THE WEST INDIES—BARBADOS

Applications are invited for the post of PROFESSOR/SENIOR LECTURER IN THE DEPARTMENT OF BIOLOGY. Duties to be assumed as soon as possible and not later than September 1, 1979.

Salary scales: Professor (in the range), BDS$36,729 to BDS$45,480 per annum: Senior Lecturer: BDS$25,827 to BDS$34,017 per annum (£1 sterling= BDS$3.99). F.S.S.U. Unfurnished accommodation will be let by the University at a rental of 10% of salary. A housing allowance of 20% of salary is payable to staff who make their own housing arrangements. Family passages. Study and Travel Grant. Detailed applications (three copies) with curriculum vitae and naming three referees should be sent direct as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

9637(A)

RESEARCH ASSISTANT

to work on the Biochemistry of Sickle Cell Disease. Salary on the Whitley Council Scale. Please apply to the Secretary, U.C.H. Medical School, University Street, London WC1E 6JJ quoting reference RA/EH. 9639(A)

SENIOR RESEARCH APPOINTMENTS

BERKELEY NUCLEAR LABORATORIES

Applications are invited for two posts of Division Head at the CEGB's Nuclear Laboratories at Berkeley in Gloucestershire. The function of the Laboratories is to provide the necessary technical and scientific support to Plant Operation, Construction, Commissioning and Forward Ordering of new plant as part of the CEGB's expanding nuclear power programme. The Laboratories have the full range of facilities expected of a major industrial laboratory.

Division Heads report directly to the Director of the Laboratories and are responsible for the initiation and effective management of a broad range of research programmes. With these two appointments, the Laboratories will be organised as five scientific divisions and appropriate supporting groups, with a total staff of 720 of whom 280 are graduate scientists and engineers.

The range of technical disciplines involved in the Laboratories research programmes is very wide and thus candidates with good academic qualifications in any of the engineering or physical sciences will be considered. More important than the candidate's technical discipline is the ability to demonstrate a proven record both of first class personal research and effective research management preferably in a major engineering-based industry. Whilst it is desirable that this experience is relevant to the nuclear energy industry, this is not an exclusive requirement. These are challenging and important posts demanding high personal qualities and a significantly long period of relevant experience.

The salary for these appointments is in a range up to £13,600 p.a. (due for review as from 1 April 1979).

Applications giving details of qualifications, experience, publications etc. should be sent within two weeks to W. H. F. Brooks, Divisional Personnel Manager, Courtenay House, 18 Warwick Lane, London EC4P 4EB. Quote Ref. **NRD1.**

CENTRAL ELECTRICITY GENERATING BOARD
RESEARCH DIVISION

9681(A)

LINCOLN COLLEGE
(University College of Agriculture)
New Zealand
LECTURER IN CROP PHYSIOLOGY

Applications are invited for the position of Lecturer in the Department of Plant Science. Applicants should have qualifications and research experience in crop physiology, preferably combining a sound knowledge of physiological principles with experience of crop or pasture production. Teaching at undergraduate and postgraduate levels will necessitate competence in such topics as photosynthesis, water relations, competition etc.

Commencing salary according to qualifications and experience. Present salary scale is: NZ$11,529 to NZ$14,250. Travel and removal expenses are reimbursed up to specified limits. New Zealand Government Superannuation is available.

Initial enquiries may be made to the following now on study leave in England: Dr P. Jarvis, Plant Physiology Department, University of Sussex, or Dr W. R. Scott, Plant Breeding Institute, Cambridge.

Conditions of Appointment are obtainable from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the College.

Applications close on April 16, 1979. 9611(A)

POSTDOCTORAL POSITION

available immediately for research on membrane phenomena associated with mediator release in allergy. Applicants should have a background in immunochemistry or biochemistry. Send curriculum vitae and three letters of recommendation to Dr A. Froese, Department of Immunology, University of Manitoba, 730 William Avenue, Winnipeg, Manitoba, CANADA R3E OW3. 9648(A)

UNIVERSITY OF GLASGOW
DEPARTMENT OF BIOCHEMISTRY
RESEARCH ASSISTANT

Applications are invited from graduates in Biochemistry, or related subjects, to work on a project concerned with Neurotransmitter metabolism in brain cell cultures.

The project is funded by the Scottish Home and Health Department, and is under the supervision of Dr R. I. Freshney, Beatson Institute, Glasgow, and Dr P. F. T. Vaughan, Biochemistry Department, Glasgow University.

The position will be for two years at a starting salary of £3,634 rising to £3,883 (Range 1B, Research and Analogous Staff scales).

Applications, enclosing curriculum vitae and the names of two referees, should be sent to Dr P. F. T. Vaughan, Biochemistry Department, University of Glasgow, Glasgow G12 8QQ, by March 2, 1979.

In reply please quote Ref. No 4364M. 9624(A)

UNIVERSITY OF LEEDS
DEPARTMENT OF CARDIOVASCULAR STUDIES

Technician in Charge required to supervise cardiological physiological techniques. The post involves administration, quality control, training of staff, and co-ordination of routine diagnostic technical investigations in two hospitals, and requires an ability to control a large technical staff (30 plus technicians) and be responsible to hospital consultants. Experience in the techniques of cardiological investigation is essential. Prior enquiry to the department is encouraged. The level of qualification looked for is H.N.C. or equivalent together with substantial relevant experience.

Salary is on the scale £5,085 to £5,517 (Grade 8b).

Salaries are presently under review.

Applications to:

Professor R. J. Linden
Department of Cardiovascular Studies
New Medical School
University of Leeds
Leeds LS2 9JT. 9674(A)

Ph.D IMMUNOLOGIST/ IMMUNO-PHARMACOLOGIST

We have a vacancy for a research graduate in the Department of Biochemical Pharmacology at our R & D Laboratories situated in Leicestershire

Applicants should preferably be qualified in immunology or pharmacology with relevant research experience to Ph D level The successful applicant will join a team of biologists investigating cellular and animal models of rheumatoid arthritis

Fisons offer a competitive salary and assistance with relocation expenses where appropriate

For an application form please write or telephone Mr Bryan Johnston, Fisons Limited, Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire LE11 0QY. Tel: 0309 66361 quoting reference number RD45/N. 9627(A)

FISONS

PHARMACEUTICAL DIVISION

Southampton THE UNIVERSITY

DEPARTMENT OF PHYSICS

Applications are invited for two LECTURESHIPS in the Department of Physics The persons appointed will be expected to play a full part in the Department's programme of teaching and research in experimental physics It is likely that preference will be given to candidates with research experience in the fields of condensed matter physics, low-temperature physics and space physics

Salary scale £3,883 to £7,754 per annum plus U S S The initial salary will depend on qualifications and experience

Further particulars may be obtained from Mrs E C P Sears, Staffing Section The University, Southampton SO9 5NH to whom applications (7 copies from United Kingdom applicants) should be sent not later than March 26, 1979 Please quote reference number 1703/A/Na 9470(A)

THE QUEEN'S UNIVERSITY OF BELFAST

RESEARCH SCIENTIST

DEPARTMENT OF MEDICINE

Applications are invited from graduates with an honours degree in biochemistry, physiology or biology, preferably postdoctoral, for a research programme into the role of gut and pancreatic hormones in diabetes mellitus. Facilities would be provided in the Department of Medicine, where multiple analytical, histological and chemical techniques are available The post is tenable for one year from April 1, 1979 or as soon as possible thereafter and may be renewed until January, 1982

Salary scale (under review) £3,885 to £5,130 with superannuation, initial placing depending on age, qualifications and experience

Curriculum vitae, giving the names and addresses of two referees, should be sent to the Personnel Officer, The Queen's University of Belfast, BT7 INN, Northern Ireland Closing date March 5, 1979 9628(A)

KING'S COLLEGE HOSPITAL

DEPARTMENT OF CHILD HEALTH

RESEARCH ASSISTANT

A graduate research assistant with experience in standard biochemical enzymatic and immunological techniques is required to join an on-going study of Collagen metabolism in liver disease in infancy and childhood This is part of a larger project being undertaken jointly by the Department of Child Health and the Liver Unit at King's investigating factors which cause chronic liver diseases in this age group.

The objective of this study is to find means of controlling collagen deposition and thereby preventing permanent liver damage.

Pay will be according to qualifications and hours.

For application form and job description, please apply to the District Personnel Department, King's College Hospital, Denmark Hill, London SE5 9RS. Tel. 01-274 6222 ext 2254. Closing date; March 1, 1979 9602(A)

AUSTRALIAN NATIONAL UNIVERSITY

Research School of Physical Sciences

FELLOW/SENIOR FELLOW—LASER PHYSICS

DEPARTMENT OF ENGINEERING PHYSICS

Applications are invited for a post of Fellow/Senior Fellow in the Research School of Physical Sciences, to provide leadership for a research group in Laser Physics which is part of the Department of Engineering Physics. The research group currently has three other (non-tenured) members of academic staff and appropriate professional and technical supporting staff. It has a well established laboratory with two neodymium laser systems producing peak powers of up to a few hundred gigawatts. Research centres on the interaction of intense laser radiation with matter. Current main research areas are the spectroscopy of, and superthermal particle generation in, laser-produced plasmas, and non-linear forces in laser-matter interactions.

The appointee will have a strong background of experimental laser-based physics. In addition to carrying out his own research he will be expected to develop and coordinate research projects which fully utilise the group's facilities and joint expertise in ways suitable to a university research laboratory whose emphasis is on basic physical science, initiate and cooperate in research projects with others in the Research School, and collaborate as appropriate with other Australian laser groups

Appointment is for five years in the first instance with the possibility of extension, after review, to retiring age, 65 years

Closing date. March 16, 1979.

Salary will be in accordance with qualifications and experience within the ranges Senior Fellow $A24,686 to $A28,773 per annum, Fellow $A18,401 to $A24,652 per annum Current exchange rates $A1 · $US1 13 · 56p.

Reasonable appointment expenses are paid Superannuation benefits are available for applicants who are eligible to contribute Assistance with housing is provided for an appointee from outside Canberra

The University reserves the right not to make an appointment or to make an appointment by invitation at any time

Prospective applicants should obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square London WC1H 0PF 9608(A)

QUEEN ELIZABETH COLLEGE

Kensington

(University of London)

LECTURER IN PHYSICS

Applications are invited for the post of Lecturer in Physics The Department, besides the traditional single-subject physics course, has undergraduate courses in medical physics and astrophysics Applicants should preferably have interests in teaching and research directed either towards the analysis and application of images formed by optical, radio or matter waves or the associated optoelectronic and solid state devices. Salary on scale £3,909 to £7,754 per annum, the starting salary depending on qualifications and experience. London allowance of £450 per annum is paid in addition.

Apply to the College Secretary, Queen Elizabeth College, Campden Hill Road, London W8 7AH from whom further particulars and an application form may be obtained.

9605(A)

AUSTRALIAN NATIONAL UNIVERSITY

CENTRE FOR RESOURCE AND ENVIRONMENTAL STUDIES

DIRECTOR

The University seeks to appoint a Head of the Centre for Resource and Environmental Studies to succeed Professor Frank Fenner, C M G , M B.E , F A A , F R S., who will retire at the end of 1979.

The Centre is designed to advance research in resource and environmental studies, to provide postgraduate training including a Master's degree by coursework, and to seek solutions to problems of resource and environmental management The present academic staff, including the Director, comprises five senior tenured staff and seven research fellows, drawn from both social and natural science disciplines.

The Director is responsible through the Vice-Chancellor to the University Council for the operation and development of the Centre and for its administration and financial management The Director's most important role is that of academic leadership, he will be expected to lead and coordinate multidisciplinary research studies within the Centre, taking a key role in developing the major projects that will at any time occupy a substantial part of the time of all staff of the Centre His professional background may be in any field appropriate to the Centre He should have a broad interest in and understanding of resource and environmental studies, and should if possible have had experience in leading multi-disciplinary team projects

The Director will also hold a Chair in the University The term of office as Director will be a matter for agreement with the University but is likely to be for five to seven years with the possibility of extension for a further period Appointment as professor is until retiring age, i e the end of the year in which the professor reaches the age of 65 years, with the option of retiring at any time after age 60.

Anyone interested in being considered for this appointment is asked to write in confidence to the Registrar, P O Box 4, Canberra, ACT 2600, Australia from whom further information may be obtained.

Reasonable appointment expenses are paid Superannuation benefits are available for applicants who are eligible to contribute Assistance with housing is provided for an appointee from outside Canberra

The University reserves the right not to make an appointment or to make an appointment by invitation at any time

9636(A)

UNIVERSITY OF ZAMBIA

Applications are invited for the post of SENIOR LECTURER/LECTURER IN THE DEPARTMENT OF PHYSICS Applicants must have a Ph D or equivalent Applicants for Senior positions must have considerable University lecturing experience Preference will be given to persons interested in Solid State Physics and Nuclear Physics Appointee will be required to teach undergraduate courses leading to a B Sc in two subjects or in Physics Salary Scale: Senior Lecturer K7,608 to K8,016 per annum Lecturer K4,956 to K7,608 per annum (£1 sterling=K1 56) The British Government may supplement salary in range £2,922 to £4,350 per annum (sterling) for married appointee or £930 to £1,968 per annum (sterling) for single appointee (reviewed annually and normally free of tax) and provide children's education allowances and holiday visit passages Family passages, baggage allowance, medical aid scheme; regular overseas leave Detailed applications (2 copies) with curriculum vitae and naming three referees to be sent direct to Registrar, University of Zambia, P O Box 2379, Lusaka, Zambia, by March 31, 1979. Applicants resident in the U K should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address.

9635(A)

UNIVERSITY OF EXETER
LECTURER IN COMPUTING SCIENCE

Applications are invited for a lectureship in the Department of Computer Science, tenable from October 1, 1979

Candidates should be qualified persons working in any area of computer science Specialisations which are of particular interest, but which are not intended to make up an exhaustive list, are data-base computation and theory, artificial intelligence, computer architecture and systems, theory of programming languages and semantics, operating systems, distributed computing and networks.

Commencing calary will be within the range £3,883 to £4,631 per annum (under review) on the scale £3,883 to £7,754 per annum (under review) The post is subject to a probationary period not exceeding three years with the prospects of permanency thereafter

Further particulars may be obtained from Miss Doreen Watson, University of Exeter, Northcote House, Exeter EX4 4QJ, to whom applications (eight copies, overseas candidates one copy) should be forwarded by March 9, 1979 Please quote reference No 3205

9659(A)

ARMAGH OBSERVATORY
ARMAGH, N. IRELAND

Applications are invited
for the post of

RESEARCH ASSISTANT

The successful candidate will work in close cooperation with the Observatory staff on spectrophotometric programmes Candidates should preferably have a Master's degree in Physics or Astronomy, but a candidate with a good honours degree in the same subjects may be considered Some familiarity with modern techniques of data reduction by computer is highly desirable

Appointment is tenable for one year from October 1, 1979, but may be renewable Remuneration according to Range 1B for University Research Assistants

Applications stating qualifications and experience and names of two referees should be received not later than May 1 1979 by the Secretary, from whom further particulars may be obtained

9668(A)

JOHN INNES INSTITUTE NORWICH
HEAD OF VIRUS RESEARCH DEPARTMENT

Applications are invited for the above position

The Department is concerned with the biochemistry and biophysics of plant viruses, mycoplasmas and their constituents, the structure and composition of proteins, the composition and function of nucleic acids, and related topics. Work in similar fields is carried out in the other three departments and collobaration between departments is encouraged.

The facilities available in the Institute are comprehensive Teaching at undergraduate and postgraduate level may be undertaken. The Institute is modern and well situated Further particulars may be obtained from the Director, Professor Roy Markham, F R S.

The post is graded as Senior Principal Scientific Officer (£10,043 to £11,300) Non-contributory superannuation

Applicants should forward curriculum vitae, list of publications and names of suitable referees to the Secretary, John Innes Institute, Colney Lane, Norwich NR4 7UH, by March 31, 1979

9667(A)

THE UNIVERSITY OF NOTTINGHAM
LECTURESHIP

in the

DEPARTMENT OF THEORETICAL MECHANICS

Applications are invited for the above appointment Candidates should be qualified in a branch of mathematics which has some relevance to engineering, for example solid mechanics, fluid mechanics, electromagnetic theory, optimisation theory, numerical analysis, statistics, control theory or applied analysis

The duties of the post will include the teaching of mathematics to Engineering students and to Honours Degree level to students in mathematics-with-Engineering

The appointment will be made within the salary scale for University Lecturers, £3,883 to £7,754 per annum (interim scales) but the appointment will be made initially within the range £3,883 to £4,382 per annum

Further particulars and forms of application, returnable not later than March 2 1979, from the Staff Appointments Officer, University of Nottingham, University Park Nottingham NG7 614

9660(A)

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF ENGINEERING
RESEARCH ASSOCIATE or ASSISTANT
for High Resolution Electron Microscopy

Applications are invited from persons who must have practical experience in both high-resolution electron microscopy and electronics, for an S R C-funded post with the Cambridge University 600 kV high-resolution electron microscope project.

The successful applicant will primarily be expected to assist external users in the operation of the microscope but will also be required to assist in the trouble-shooting and repair of a variety of electronic items and to construct simple electronic circuits Some standby/shift work may occasionally be necessary

Tenure 18 months from April 1, 1979

Pensionable salary in the range of £4 631 to £6,080 per annum, depending on age and experience

Applications (two copies), including a curriculum vitae, and the names of two referees should be sent to the Secretary, Faculty of Engineering, Cambridge University Engineering Department, Trumpington Street, Cambridge CB2 1PZ by February 28 1979

9666(A)

Head of Physical and Analytical Services Unit

The continuing expansion of the Medicinal Research Centre has led to the creation of a Physical and Analytical Services Unit, to undertake wide-ranging analytical and physico-chemical studies relating to our programmes in medicinal chemistry during all phases of research and development.

The Unit Head will be required to staff and equip this function and to contribute in the design of new Laboratories A staff of some twenty could ultimately be envisaged, encompassing spectroscopy, elemental microanalysis, chromatography and general analysis

Candidates should possess at least an appropriate first degree and have some years' relevant experience of analytical chemistry, preferably of pharmaceutical substances Considerable store will be placed upon a candidate's management potential and the ability to align the service closely to the needs of the research programmes through an outgoing and innovative approach

If you wish to be considered for this new post, please write, outlining your career to-date, to **John Atkinson,**

Site Personnel Manager,
Beecham Pharmaceuticals,
Medicinal Research Centre,
The Pinnacles,
Harlow, Essex. 9701(A)

Beecham
Pharmaceuticals

ANATOMIST
BROWN UNIVERSITY

invites applications
and nominations for a position as

INSTRUCTOR or ASSISTANT PROFESSOR

to teach human morphology and to conduct research in any area of morphological sciences. Applicants should have a doctoral degree and a demonstrated competence and enthusiasm for the teaching of the gross, functional anatomy of man in the perspective of his evolutionary and embryological development and in the focus of its clinical relevance Candidates with a productive research programme in comparative anatomy, primatology, embryology, neuroanatomy and other aspects of the morphology of whole organisms will be especially welcome Starting date July 1, 1979 Send before March 1, 1979, a curriculum vitae, description of teaching and research interests, and three letters of recommendation to Dr G E Erikson, Division of Biology and Medicine, Box G , Brown University, Providence, R I 02912 An Equal Opportunity/Affirmative Action Employer

9665(A)

ST THOMAS' HOSPITAL MEDICAL SCHOOL Academic Unit in Immunology Research Technician (MLSO/SMLSO) or Graduate Research Assistant Required from April 1979 for work on the Immunology of Blindness Experience in Cell/Tissue culture an advantage Usual salary scale (Whitley Council/University) according to qualifications/experience Applications quoting names of two referees to Dr D C Dumonde, Director, Academic Unit in Immunology The Rayne Institute, St Thomas' Hospital London SE1 7EH (Tel 01-929 9292 ext 3186)

9685(A)

UNIVERSITY OF EDINBURGH
LECTURER IN CLINICAL CHEMISTRY

Applications are invited from medical graduates and from science graduates holding an Honours Degree in Biochemistry or Chemistry or one of the related sciences, for the post of Lecturer in the Department of Clinical Chemistry which is situated in the Royal Infirmary of Edinburgh The department is responsible for providing a wide range of diagnostic services in clinical chemistry, is involved in teaching at several stages in the medical curriculum, and has good facilities for research and development The department is registered as a training laboratory for postgraduate professional qualifications. Arrangements can be made with Professor L G Whitby (031-229 2477, ext 2319) to visit the department

Salary on the scale £4,336 to £7,683 for a medically qualified Lecturer, or £3,883 to £7,754 for a non-medical Lecturer, and the successful applicant will be recommended for the award of an Honorary Contract by the Lothian Health Board.

Applications should be submitted by March 10, 1979, together with the names of two referees, to the Secretary of the University, Old College, South Bridge, Edinburgh EH8 9YL, from whom further particulars may be obtained Please quote Reference 1012.

9690(A)

FACULTY POSITIONS OPEN

College of Agriculture and Food Sciences
KING FAISAL UNIVERSITY
Hoffuf, Saudi Arabia

Inquiries invited from qualified applicants at all academic levels to teach courses in the following fields:

- Agricultural Economics
- Animal Science
- Animal Ecology
- Agronomy
- Agricultural Engineering
- Dairy Science
- Bee Keeping
- Horticulture
- Agricultural Extension
- Water Resources and Climatology
- Plant Pathology
- Home Economics and Nutrition
- Poultry Science

Prerequisites: Masters or equivalent degree; practical and/or teaching experience. Preference will be given to Arabic speakers.

Language of instruction is English.

Positions are available starting September 1979; interviews scheduled with the Dean in the United States and Great Britain during April/May 1979.

Salary Ranges

Rank	*Gross monthly salary in Saudi Riyals*	*Approx annual (sterling equivalent)*
Professor	SR 7,195 to SR 9,595	(£12,983 to £17,314)
Associate Professor	SR 6,435 to SR 8,185	(£11,612 to £14,770)
Assistant Professor	SR 5,245 to SR 7,765	(£9,466 to £14,012)
Lecturer	SR 3,280 to SR 4,530	(£5,919 to £8,174)

Benefits: Free furnished accommodations; substantial educational allowance for up to four children; air fare to and from Saudi Arabia once a year for husband, wife and two children plus 60-day paid summer holiday; no Saudi income tax.

Send complete curriculum vitae stating preference of course and names, addresses and telephone numbers of three references to:

Dr H. M. Elarosi, Dean
College of Agriculture and Food Sciences
King Faisal University

at the appropriate addresses below:

Post Office Box 2
Sunbury-on-Thames
Middlesex TW16 5JP, England;
c/o Saudi Arabian Educational Mission
2223 West Loop South, Suite 400
Houston, Texas 77027, U.S.A.

9700(A)

SHEFFIELD AREA HEALTH AUTHORITY (TEACHING)
SOUTHERN DISTRICT (TEACHING)

GROUP DEPARTMENT OF HAEMATOLOGY

State registered M.L.S.O.
or
Junior B M.L.S.O.

Applications are invited for the above post from State-registered M.L.S.O's or graduates with a Science Degree which should include as a principle subject one or more of the following:— Biochemistry, biology, chemistry, microbiology, physics, physiology or zoology, or from persons who possess two science 'A' levels, preferably chemistry and biology.

This is a career post, commencing salary within the range of £2,370 and £3,015, depending on academic qualifications.

Applications stating age, qualifications (and the names and addresses of 2 referees to the District Personnel Officer, Floor B, Hallamshire Hospital, Glossop Road, Sheffield S10 SRX 9641(A)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF RADIOTHERAPY
(Cookridge Hospital)

RESEARCH TECHNICIAN

(Grade 3 £2,688 to £3,060)

Currently required to assist experimental studies of the effect of drugs on the chromosones of normal and cancer cells.

Suitable applicants will have formal qualifications and appropriate laboratory experience in the biological sciences Applications stating age, qualifications, experience and the names and addresses of 2 academic referees should be sent to Professor C A F Joslin, University Department of Radiotherapy, Regional Radiotherapy Centre, Cookridge Hospital Leeds LS16 6QB (Tel Leeds 673411 Ext 215) from whom further details may be obtained. 9673(A)

STUDENTSHIPS

UNIVERSITY OF WARWICK
POSTGRADUATE RESEARCH
in
ENVIRONMENTAL
MICROBIOLOGY

Applications are invited for research studentships in the Department of Environmental Sciences, tenable from October 1, 1979 leading towards a Ph.D. degree in the fields of cellulose degradation, stability of bacterial drug resistance factors, inorganic nitrogen metabolism in facultatively anaerobic thiobacilli and pseudomonads, bacterial oxidation and leading of metal sulphides by mesophilic and thermophilic iron bacteria, colonisation of antifouling surfaces by bacteria, soil quality and bacterial colonisation of soil particles, microbial polymers and soil aggregation, microbial dehalogenases, rhizosphere/root region microbiology Applicants should have or expect to gain this summer, a good honours degree in Microbiology, Biochemistry or a related subject. Enquiries to Dr J H. Slater, Department of Environmental Sciences, University of Warwick, Coventry CV4 7AL 9625(F)

AGRICULTURAL
RESEARCH COUNCIL
LETCOMBE LABORATORY
A.R.C. RESEARCH
STUDENTSHIP

APPLICATIONS are invited for an A R C Research Studentship tenable for three years from October 1, 1979 at the Letcombe Laboratory for research on the effect of soil conditions on root growth and crop yield

The student will work on the effects on cereals of either water shortage or waterlogging

Applicants must have, or be likely to obtain, at least Upper Second Class Honours and will be expected to register for a higher degree

Further details and application forms from the Secretary, Agricultural Research Council, Letcombe Laboratory, Wantage, Oxon, OX12 9JT Closing date for applications March 12, 1979 9672(F)

UNIVERSITY OF ABERDEEN
FACULTY OF MEDICINE
RESEARCH STUDENTSHIPS

Applications are invited from recent first or upper second class honours graduates in a biological science subjects, and from those who expect so to graduate in 1979, for research studentships established by the Faculty of Medicine from its Medical Endowment funds Successful applicants will study for the degree of Ph D on an approved project in one of the departments of anatomy, bacteriology, biochemistry, bio-med physics and bioengineering, developmental biology, pathology, pharmacology or physiology commencing in October 1979

The value of the studentship is £1,610 p.a. (for students living away from home) with payment of tuition fees and other allowances

Application forms and further particulars of the projects may be obtained from D. K Yule, University Office, Regent Walk, Aberdeen AB9 1FX, to whom applications should be submitted by March 16, 1979.

9702(F)

ROTHAMSTED
EXPERIMENTAL STATION
Harpenden Herts AL5 2JQ
PHYSICS IN AGRICULTURE
A.R C. Research Studentship
from October 1979

Applications are invited from graduates or expected graduates in physical science for an A.R.C Research Studentship The projects would include aspects of soil water physics and water movement in plants, with reference to the movement and availability of water in agricultural soils.

A first or upper-second class honours degree or an equivalent qualification is required The successful candidate will be registered with a University for a Ph D

Applications, giving the names of two referees should be sent by March 12 to Dr W Day, Physics Department, from whom further particulars may be obtained. 9645(F)

EAST MALLING
RESEARCH STATION
AGRICULTURAL
RESEARCH COUNCIL
STUDENTSHIP

Postgraduate studentship tenable from October 1, 1979, for 3 years for research leading to higher degree on one of the following projects

Water and mineral uptake by secondary thickened tree roots,

or

Integrated control, ecological effects of pesticides on the hop-damson aphid and its natural enemies

First or Upper-second Honours degree essential.

Further details and application form from the Assistant to the Secretary, East Malling Research Station, Maidstone, Kent, ME19 6BJ 9680(F)

SYMPOSIA

FIFTH EMBO ANNUAL SYMPOSIUM NUCLEIC ACID-PROTEIN INTERACTIONS

October 12–15 inclusive

EMBL Heidelberg

In addition to members of the Organising Committee invited speakers include:

J. Abelson (La Jolla); T. Bickle (Basel); H. Bujard (Heidelberg), P. Chambon (Strasbourg); R. Flavell (Amsterdam); C. Guthrie (San Francisco), S. Harrison (Harvard); C. Helene (Orléans), H. Hoffmann-Berling (Heidelberg); A. Landy (Brown); A. McPherson (Pennsylvania); M. Ptashne (Harvard); M. T. Record (Madison); H. Saedler (Freiburg); A. Schmitz (Geneva), J. Sperling (Rehovot), G. Stöffler (Berlin); F. W. Studier (Brookhaven); E. Vinuela (Madrid); J. C. Wang (Harvard), W. Wintermeyer (Munich); C. Yanofsky (Stanford).

The eight Plenary Sessions will cover Model Systems, Viruses; Chromatin; Polymerases; Regulatory Signals on DNA; Cleaving, Processing and Modifying Nucleic Acids; Ribosomes and tRNA; New Frontiers.

The Symposium will be held at the European Molecular Biology Laboratory Heidelberg with registration on Thursday, October 11. Plenary sessions will be held in the mornings and afternoons of October 12-15 inclusive. The Symposium banquet will be held on the evening of October 15. Poster sessions will be arranged; applicants wishing to present a poster should submit a title and an abstract of 1-2 pages. Participants will be accommodated in two hotels on downtown Heidelberg, and transportation to the meeting will be arranged.

The registration fee is DM 80.00 and includes daily lunches and the banquet but neither hotel charges nor evening meals.

Applications including a curriculum vitae should be sent to Dr. J. Tooze, Executive Secretary, European Molecular Biology Organisation, Postfach 1022.40, 69 Heidelberg 1, FRG, by May 31, 1979. Since the number of participants is limited to 200 the Organising Committee will notify those who have been accepted in August.

Organising Committee: D. Crothers (Yale); J.-P. Ebel (Strasbourg); A. Klug (Cambridge); C. Kurland (Uppsala, Chairman); J. Miller (Geneva); H. Schaller (Heidelberg); J. Tooze (Heidelberg, Secretary); C. Weissmann (Zürich). 9616(M)

STRAW DECAY SYMPOSIUM

April 10, 11, 1979

HATFIELD POLYTECHNIC
HATFIELD

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A residential Symposium on "Cell Electrophoresis: Techniques and Clinical Applications" will be held in Bristol, U.K., from June 12-15, 1979.

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Short communications (10 mins oral, or poster display) are invited A Registration Fee of £10 will be charged

Further details may be obtained from Dr A W Preece, Oncology Research Unit, Radiotherapy Centre, Bristol BS2 8ED (Tel 0272 22041 extn 2469), or from Dr D Sabolovic, I N.S E R M, Unité de Cancérologie Expérimentale et de Radiobiologie, Vandoeuvre-lès-Nancy, France 54500 (Tel 83 55.52 01) 9675(M)

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CLOSING DATE. April 18, 1979. 9664(E)

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9612(E)

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9698(D)

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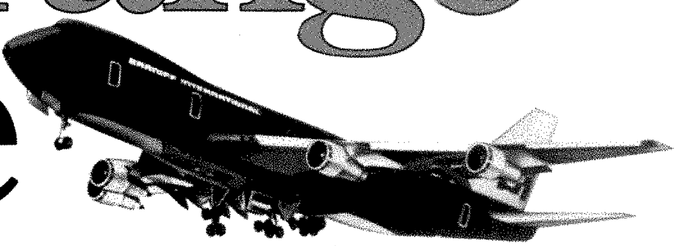

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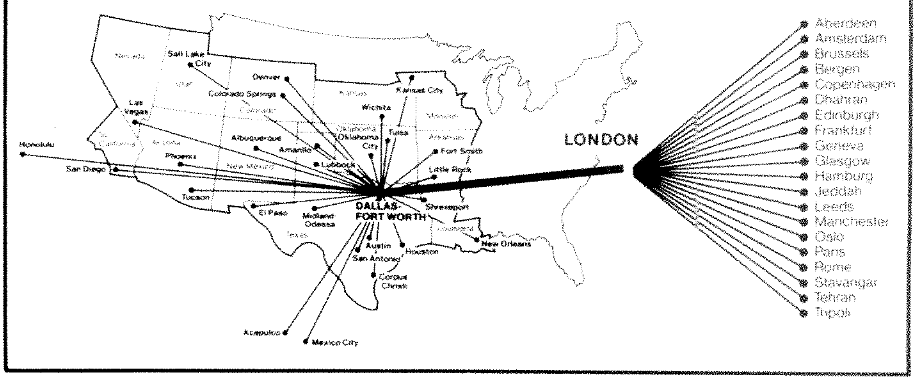

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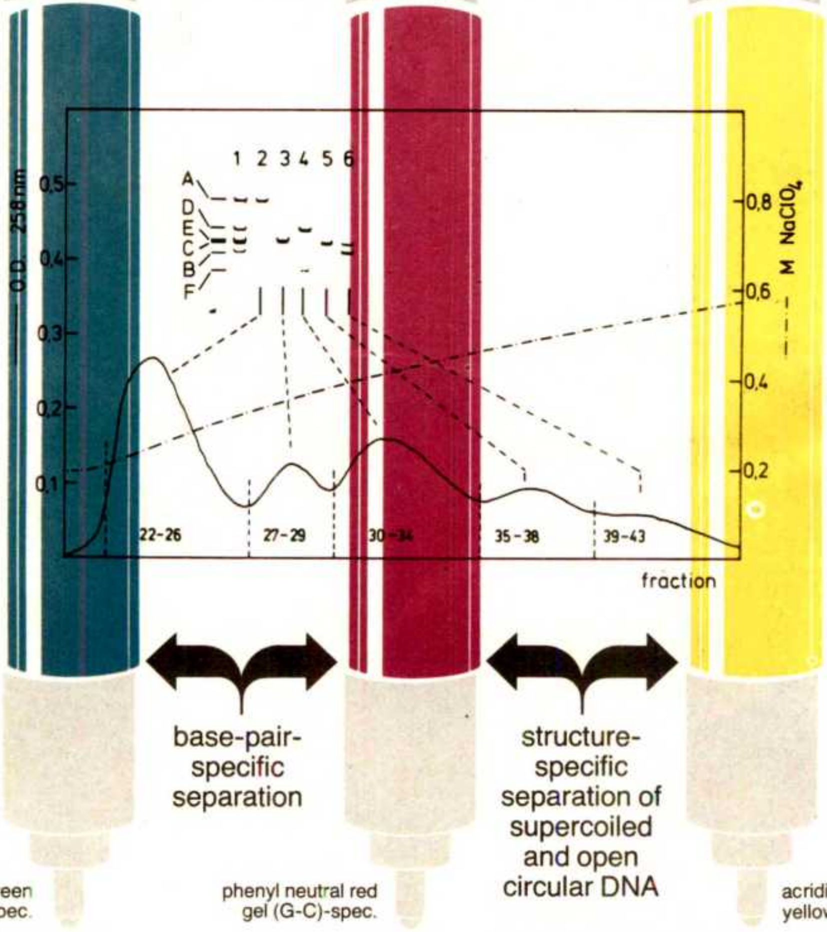

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Cover picture

Shoemaker at work, by the Eurcharides Painter (early fifth century BC). For more on magnetic field determinations using ancient pottery, see pp. 643 and 644.
[Photo: Ashmolean Museum, Oxford, UK]

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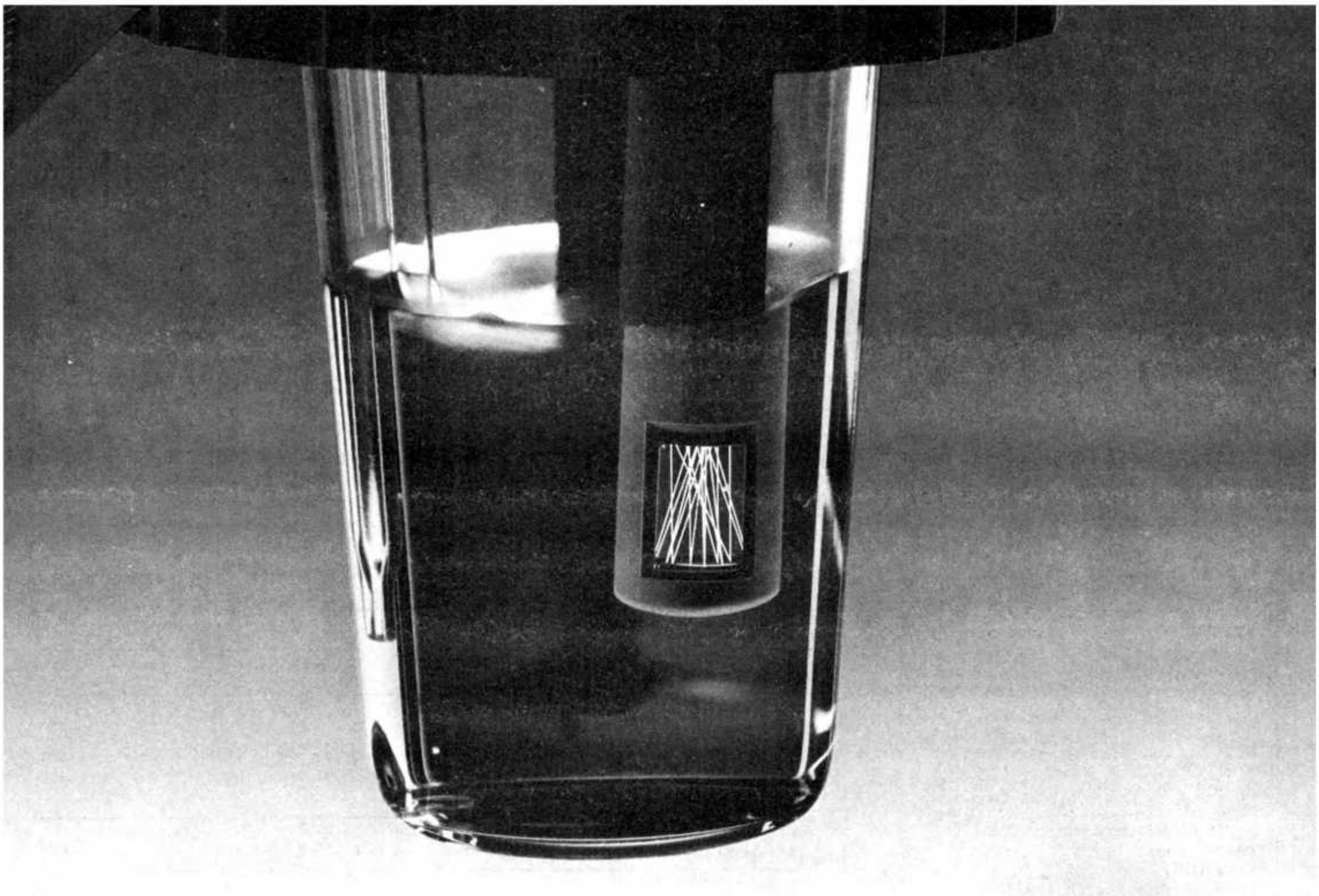

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nature

22 February 1979

Industrial innovation in the UK—a first step

Over the past few years, Britain's faltering industry has come in for much attention from a variety of official and semi-official bodies. There has been the establishment of a National Enterprise Board and several discussion documents on the engineer and his relation with universities, industry and government, culminating in the Finniston Committee of Enquiry. The Science Research Council has taken a new range of initiatives such as the Co-operative Awards in Science and Engineering scheme, Total Technology courses and Teaching Companies. Now from the very centre of government, the Cabinet Office, comes a paper *Industrial Innovation* (HMSO, £1) produced by an unnamed working party, chaired by Dr A. Spinks (ICI), for the Advisory Council for Applied Research and Development (ACARD). The working party had a brief to consider ways in which conditions for innovation in British industry might be improved and to make appropriate recommendations for Ministerial action.

'Innovation', in current usage, means much more than just invention. It encompasses improvements to present products and processes as well as the introduction of new ones, and innovation does not just comprise what goes on in the R & D laboratory; it includes all the activity that goes into getting the new or improved commodity widely accepted. Some relatively unspectacular activities such as making car doors that shut with a satisfying click, or mechanical devices that break down less frequently than their competitors may be much more profitably innovative than no end of ultra-high technology. Identifying niches that no-one else has explored, be they relatively humble in technological terms, may bring major rewards. Maybe a slower but much cheaper propellor plane would be a better project for the 1980s than another supersonic jet transport.

It is not only Britain that is seriously worried about its industry's record. The United States is equally concerned about overseas competition from Japan and Western Europe, even with the built-in help of an enormous and affluent domestic market, many US industries are finding themselves being undermined. At present an enormous nationwide effort is in train to come up with some policy options for President Carter. By contrast the British document a mere 26 pages in length looks slim indeed, and sensible as many of the suggestions are, they sadly lack the sort of detailed analysis, case studies and reference to give the paper the weight which will impress ministers and their advisers.

The report lists, as obstacles to innovation in Britain, continual changes in the economic environment, lack of incentives for managers and entrepreneurs, the burden of legislation, overmanning, falling industrial productivity, emphasis on short term returns, lack of qualified manpower and technological ignorance in board rooms. The working party, in response to this, recommends a variety of corrective actions—a tax structure more favourable to investment and to small businesses, more encouragement to Research Associations, strengthening university/industry relationships, pressure on nationalised industries and government procurement agencies to support their suppliers, notably small technologically based industries, and so on.

Few would argue either with the analysis, brief as it is, or that the measures suggested were inappropriate, but even so the working party has concentrated its attention heavily on tactics, rather than on strategy. We urgently need to know the answer to questions such as what sort of industrialised nation does Britain wish to become; whether it will incorporate into an industrial policy any considerations of technological interaction with the developing world; whether our present educational system, at secondary as well as at tertiary levels, does enough to make students aware of the importance of less-glamorous industry to our survival as a trading nation; whether there are important environmental, 'public-interest' and health-and-safety issues which ought to be raised in any discussion on industrial improvement; whether there is a 'rational' level at which Britain should spend on R & D.

ACARD have made a useful modest step forward in confronting the question of industrial innovation. It is to be hoped that they will not regard the job as finished. ☐

Science has a word for it: We recently asked for examples of words or phrases from the world of science that could be given a much wider currency. Some outrageous examples were received of the misuse of words: from Stonybrook came a memorandum describing the university as a 'multi-ethnic and bi-sexual community'; from NASA came 'inhibit time advance' as an instruction to stop a computer programme, and 'versatilify'. But the prize of £10 goes to Donald Higby, of Roswell Park Memorial Institute, Buffalo, for a string of terms from cancer medicine including 'metastasize' as in 'I will have to metastasize to the conference' and 'dose-limiting toxicity' in 'I've reached the dose-limiting toxicity in my relation with Dr X'.

Italian Commission covered up Séveso delays

Italy's official report on the Séveso accident leaves out some important information, writes **Alastair Hay**

THE 412 page Italian Parliamentary Commission Report on the accident at Séveso in Italy in July 1976—an accident in which tetrachlorodibenzodioxin (dioxin) from ICMESA's trichlorophenol reactor was discharged over a populated area—should have been the definitive report on the episode. But documents in *Nature's* possession indicate that the claims of Givaudan—a subsidiary of F. Hoffmann la Roche and the owner of the reactor—are more accurate than those of the commission's report.

The bitter and continuing disagreement centres on the sequence of events between 10 July 1976, when the Séveso accident occurred and 24 july when evacuation of the dioxin-contaminated area was ordered. Givaudan claims that it was responsible for urging the authorities to evacuate the area. But the commission's report assigns a far more important role to Italian scientists in recognising the danger. Another report however, that of the Lombardy Regional Government supports Givaudan's claims and indicates that the Italian Parliamentary Commission omitted some vital evidence in its report.

Few would disagree that the two-week delay in ordering the evacuation of residents may have had serious consequences for health. The Italian authorities claim that the delay was the result of a lack of information on the extent of the dioxin contamination. According to the Parliamentary Commission report it was not until the evening of 23/24 July that "positive evidence of the presence" of dioxin was obtained from the results of analyses performed by the Italian laboratories—the Superior Health Institute in Rome and the Provincial Laboratory of Hygiene and Prophylaxis (PLHP) in Milan.

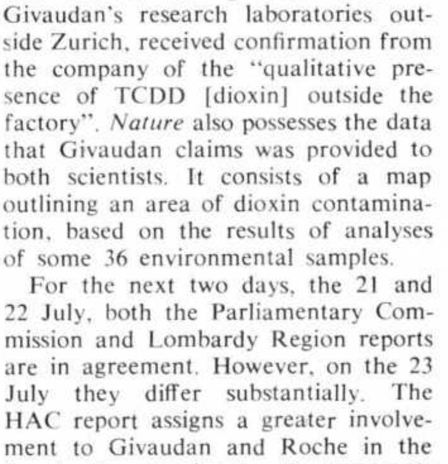
The full version of the commission's report—only recently made available to interested parties—differs substantially from the summary made available to the press by the Italian authorities when the report was presented to the Italian Parliament in June 1978. The press version implies that the Italian laboratories found dioxin and that their results were used as the basis for ordering the evacuation.

The account in the full report, however, says that two Italian scientists, Drs Ghetti and Cavalloro, flew to Givaudan's laboratory at Dubendorf, near Zurich on 19 July and there received confirmation that dioxin had been discharged into the atmosphere at Séveso. They immediately returned to Italy and duly informed the various authorities. Meetings were held in the days following, and arrangements made for monitoring the situation. It was not until a meeting on 24 July, under the chairmanship of the Health Assessor Dr Rivolta, that the evacuation was ordered. The Italian laboratory results were presented at the meeting, says the report, and confirmed by the Givaudan representative, Dr Vaterlaus.

However, neither the commission nor the press release versions are supported by official reports of the Health Assessor's Council (HAC) of the Regional Board of the Lombardy Region. One controversial issue is the question of whether the ICMESA management notified the appropriate authorities about the accident and its potential seriousness on 10 July. The main HAC report says that it did. All reports confirm that it was the 15 July when the first order was posted by the local mayor to warn the population about the contamination and banning the "consumption of fruit and vegetable products" grown in the vicinity of the factory.

On 18 July, says the HAC report Dr Cavalloro, Director of the PLHP in Milan, suspected the presence of dioxin in the discharge from the reactor. His suspicion was confirmed by ICMESA although the parent company, Givaudan, says the report, "does not admit that there is dioxin; it allows this to be understood". Under the entry for 20 July, the HAC report says that on the previous evening Drs Cavalloro and Ghetti, during their visit to Givaudan's research laboratories outside Zurich, received confirmation from the company of the "qualitative presence of TCDD [dioxin] outside the factory". *Nature* also possesses the data that Givaudan claims was provided to both scientists. It consists of a map outlining an area of dioxin contamination, based on the results of analyses of some 36 environmental samples.

For the next two days, the 21 and 22 July, both the Parliamentary Commission and Lombardy Region reports are in agreement. However, on the 23 July they differ substantially. The HAC report assigns a greater involvement to Givaudan and Roche in the Lombardy Regional Government's deliberations than does the commission's report. It says that on 23 July a representative of Roche arrived at an extraordinary meeting of the Provincial Health Council in Milan where various Italian scientists with an interest in Séveso were considering



Child exposed to dioxin

0028-0836/79/0277—0588$01.00

the measures to be adopted and was forbidden to speak. Later that day at another meeting—of the local administrators of the Séveso region—the representative, Dr Guiseppi Reggiani, Director of Clinical Research for Roche, stressed to the Health Asessor, Dr Rivolta, the necessity and urgency of evacuating the population at Séveso exposed to the highest concentrations of dioxin. Rivolta asked him to put his concern in writing. The report says that this request was complied with on 24 July in a letter (signed by Givaudan's president, Dr Guy Waldwogel) At the same time, the report adds, a map of the polluted area was handed over, with the first quantitative data on the pollution released by Givaudan. The map contained the results of analyses of 44 environmental samples The Lombardy Region report suggests therefore, that Givaudan and Roche played a far larger role in documenting the pollution at Séveso than the Parliamentary Commission gives them credit for.

On the 19 July however, Givaudan claims that the two Italian scientists Ghetti and Cavalloro, who visited the company's laboratory near Zurich, received most of this data—albeit from some 36 environmental samples. If this is true why does the Parliamentary Commission report not mention it? (*Nature* has ascertained that this information was presented to the commission during a six hour meeting) and why had the Italian authorities not even considered an evacuation by 23 July when they knew of the dioxin contamination on 20 July?

The Parliamentary Commission report admits that the laboratory facilities and expertise required to assess dioxin contamination at Séveso were not available in Italy. This is one reason why Ghetti and Cavalloro were provided with the necessary dioxin standards when they visited Givaudan on 19 July. Dr Cavalloro declined to tell *Nature* the number of measurements his laboratory had made between 20 July and 11.00 p.m. on 23 July. Asked about the differences between the reports of the Parliamentary Commission and Lombardy Region he felt he was not competent to answer and that as it was a political question it should be directed to the Lombardy Regional Government. He and his colleagues, he said, were "only technicians".

Signor Cesare Golfari, President of the Lombardy Regional Government told *Nature* that he thought the Parliamentary Commission Report was "good for the Regional Government". In his opinion, it gave an adequate account of events at Séveso. Roche, too, welcomes the overall objectivity of the commission's report—the commission was eqaully scathing of Roche, Givaudan and many of the Italian authorities. A spokesman for Roche, however, described the commission's report as telling a "twisted tale". He said that he "certainly" knew that the commission's charge that Givaudan had not informed its workers at ICMESA about the hazards of dioxin was true. He also said that all but 6 or 8 of some 6,000 civil claims for damages against Roche had been settled and conceded that the lawyers had recommended prompt settlement of claims to avoid a higher bill if the criminal court case went against the company.

The criminal prosecution is in the hands of the Italian Judiciary. The court will consider whether or not Givaudan was negligent in its maintenance of the trichlorophenol reactor at Séveso, and whether it could have informed people more quickly after the accident of the danger they faced. The hearing is unlikely to take place for some time however, for the judge originally appointed to deal with the case has recently been promoted and his successor will need some time to familiarise himself with the background to the case.

Until the court hearings are available, the Parliamentary Commission report will remain the most comprehensive appraisal of the accident at Séveso. Britain's Health and Safety Executive (HSE), is to have the document translated. When that is available much of what happened at Séveso will be available for public scrutiny outside Italy. ☐

Genetic manipulation: Britain may exempt "self-cloning"

Britain's Genetic Manipulation Advisory Group will meet this week to reconsider issues which have been bothering the group for some time—particularly the definition of genetic manipulation and whether it should include "self-cloning" experiments.

These are experiments in which the genes of a bacterium are cloned within the bacterium itself—such as *E. coli* within *E. coli*—which are already exempted from control under the revised US National Institutes of Health guidelines.

GMAG has been under considerable pressure from bacteriologists—and lately the Royal Society (see *Nature*, 15 February, page 509)—to relax its control of such experiments, which form a substantial proportion of potential recombinant DNA research; and now it appears to be responding. Even if GMAG decides in favour of a strict definition of recombinant DNA experiments which includes self-cloning, it is felt that it will move to exempt the experiments from categorisation.

At its last meeting GMAG decided "in principle" to adopt the new risk assessment scheme devised by Sydney Brenner—but only for a trial period of about one year. Four proposals for experiments have already been received for consideration by risk assessment, including one application for downgrading, and two have already been assessed. In these cases the categorisation was the same as it would have been under the phylogenetic Williams guidelines, so there was no conflict, but members of GMAG are known to be worried about cases where there is disagreement. It is felt that much is yet to be learned about real risk factors, and that much more research on them needs to be undertaken before risk assessment can be firmly established.

Meanwhile the "technical panel"—which will make the assessments and advise the Medical Research Council and Health and Safety Executive on risk research that needs to be undertaken—is being established under the chairmanship of Professor Peter Walker of the University of Edinburgh, a man who has been not uncritical of excessive control of recombinant DNA research.

The technical panel will have some nine "core" members, including representatives of GMAG and outside experts, and will also co-opt special expertise when necessary.

This week's meeting of GMAG will be a particularly interesting one for it may be the first at which the deeper views of its seven new members (out of a total of 19) will be tested. The last meeting was concerned with a detailed discussion of the risk assessment scheme, and like many of the regular GMAG meetings it failed to address—or at least reach decisions on—certain matters of principle such as the basic definition of its area of competence. Hence this week's extraordinary meeting, called specifically to address these questions.

If the meeting reaches its conclusions rapidly, GMAG will publish a short document for researchers outlining its new policy. A full note will be issued in due course.

Robert Walgate

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MPs accuse health officials of unconcern over risks and benefits of DNA research

BRITISH members of Parliament last week accused officials of the Department of Health and Social Security of displaying an "active disinterest" (*sic*) in the possible health risks of research involving the use of recombinant DNA techniques.

In particular, the MPs expressed concern that a non-specialist assessor who sits on the Genetic Manipulation Advisory Group on behalf of the DHSS should represent the UK on an ad hoc group of experts which has drawn up a draft directive on the regulation of genetic manipulation experiments for the European Economic Commission.

These concerns were voiced at a meeting last week of a subcommittee of the House of Common Select Committee on Science and Technology which is currently holding a series of public meetings on genetic engineering in the UK.

Mr C. M. Regan, under-secretary responsible for public and environmental health at the DHSS, told the MPs that because recombinant DNA techniques essentially involved research processes, their safety was a matter for the Department of Education and Science and the Health and Safety Executive rather than DHSS. Although DHSS had an assessor on GMAG, the public health aspects of possible hazards was therefore "not a matter which falls to us directly".

The MPs accused the DHSS officials of taking an unimaginative approach, both to their responsibilities for protecting the public from unpredictable hazards of the research, and to the possibilities that the research promised for the production of drugs such as insulin.

Mr Ted Leadbitter, who had last week accused DES officials of using "under-secretary language" in giving evasive replies to the members' questions, referred to the memorandum submitted to the subcommittee by the DHSS as a "pathetic document" revealing what he called an "active disinterest" in the matters under review.

Mr Leadbitter's wrath descended in particular on Dr T. J. B. Geffen, a senior principal medical officer at DHSS who represents the department on GMAG, but who told the committee that he has no particular experience or training in the techniques being discussed, and that his role was essentially that of providing a channel of communication between the department and GMAG.

Dr. Geffen, who was a hospital physician before joining the DHSS, told the MPs that he did not feel this role required any particular expertise, and that he felt there was no need for the DHSS to employ anyone with such experience since advice on technical matters relating to advances in DNA research could be obtained from consultants.

Given Dr Geffen's "relatively small" contribution to GMAG, the MPs therefore wanted to know why he had been chosen to represent the UK on an expert committee which had prepared draft directives on genetic manipulation for the EEC. They were not satisfied with the answer that the word "expert" in such a context meant someone with broad experience in the matter under discussion.

Mr. Arthur Palmer, chairman of the select committee, said that he felt the DHSS was taking a negative approach to the question of both the risks and the benefits of recombinant DNA research, and that the committee would try to put questions to Mr David Ennals, Secretary of State for Health, "to see if this policy of detachment is the best one".

David Dickson

Forest destruction must be halted, says report

THE Earth's forests are now decreasing in size annually by at least 11 million hectares—the area of Bulgaria—according to the latest Worldwatch report. It warns that man's destruction of tropical forests "is taking an enormous economic and environmental toll".

The study's author, Erik Eckholm, points out that the "fundamental importance of forests to human well-being" is generally ignored by those who analyse the basic needs of the world's poor to reduce the forest deprivation now suffered by many". (The Worldwatch Institute is doing its best to cure the blindness: the very first Worldwatch paper was Eckholm's 'The Other Energy Crisis: Firewood'.)

Information about the extent and condition of the world's forests is remarkably scanty, government statistics are frequently inaccurate and "sometimes doctored outright", the report says. But Eckholm estimates that approximately 20% of the Earth's land is now covered by dense forest—that is about half of the area that would be forested in the absence of man. Another 10% of land is "open woodland".

The forest area of North America and Europe is roughly stable. Almost all the loss is occurring in the humid tropical forests of Latin America, Asia and Africa, where Eckholm says the crude deforestation figures greatly underestimate the damage because "they reflect neither the massive degradation of timber and other biological resources occurring within many still-standing forests nor the severe depletion of open woodland and countryside vegetation that currently blights most third world countries".

The three principal causes of deforestation are, in order of importance, the unplanned spread of agriculture, firewood collection for cooking, and indiscriminate timber harvesting. Tree planting makes up only a small fraction of the loss.

"As a result the real prices of wood and wood products are likely to rise substantially over the coming decades . . .", Eckholm writes. "Meanwhile the continued loss of forests will accentuate the environmental costs of denudation already apparent in many countries—erosion, desertification, siltation, flooding and the extinction of species." And, he adds, deforestation may rival fossil-fuel burning as a source of atmospheric acrbon dioxide.

Although evidence for the economic consequences of deforestation is extremely scarce, Eckholm argues that it has been a major driving force behind the past decade's worldwide inflation. The price of wood has been rising in real terms by 4% a year during the 1970's, he estimates—and the increases have been most severe in the third world.

"A simple board costs twice as much in Pakistan as in the United States though the income of the average American is 46 times that of the average Pakistani", Eckholm reported. Families in some West African cities spend ¼ of their income on firewood.

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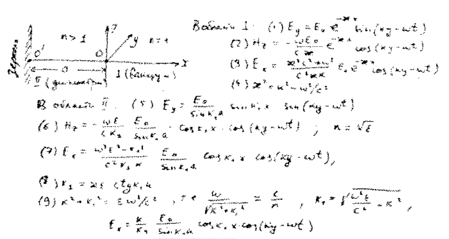
But the study concludes on an optimistic note. International aid agencies and politicians in developing countries are becoming more aware of the need to protect and manage forests. And the dismal record of tree planting in the third world—millions of young trees uprooted or nibbled to death by livestock—is showing signs of improvement in places where the "community forestry" approach is adopted. This means giving villagers the means to meet their own requirements and protect their own growing trees, Eckholm says, rather than imposing planting programmes in which they have no say—as has happened too often in the past.

The astonishing success of forestry in post-revolutionary China is the best known example of this philosophy. But Eckholm says the "success story of the 1970's" is South Korea. There a strong national commitment, combined with new village-level forestry associations, has covered one-third of the country with trees in the past decade and nearly solved an acute firewood crisis.

Worldwatch Paper 26, 'Planting for the Future: Forestry for Human Needs', is published by the Worldwatch Institute, Washington DC, at $2.

A day in the life of Yurii Orlov



A jailed dissident is keeping up his scientific work in a Soviet labour camp.

Vera Rich reports

Yurii Orlov, founder-member of the Moscow Helsinki monitoring group, was arrested two years ago this month. Unlike Academician Andrei Sakharov, who has virtually given up scientific work to devote himself to human rights, Dr Orlov, until the moment of his arrest, considered himself a scientist. Indeed, his letters from prison (which reached *Nature* recently) indicate that he is still attempting to pursue his research.

Until his trial last May, Orlov was held incommunicado. After his trial he was permitted to write the usual two letters per month, and immediately began to request writing materials and scientific texts. "I'd like to have Landau's quantum mechanics", he wrote to his wife in July, "it's lying on the shelf".

In the same letter he reports on his work in progress. "I'm constantly doing scientific calculations. . . . As you know, I've already completely finished one paper on logic, when I've copied it out, I shall send it officially to the Landau Institute of Theoretical Physics, to the Director."

However, two papers written during his pre-trial confinement had been confiscated. The next letter suggests, obliquely, that he was contemplating a hunger-strike if they were not returned.

Three weeks later, he was still anxious about the fate of these papers, and further perturbed that his paper on logic had not reached the director of the Landau Institute. But a long postscript to be passed on to his scientific friends outlined his latest train of thought. "While I was in quarantine, I did a small calculation for myself. I considered whether it was possible, by passing a beam of light along a wave-guide (i.e. with total internal reflection), to obtain beyond the limits of the wave-guide, in vacuo, an electromagnetic wave extended to a fairly great distance."

But the conditions for such quiet scientific reflection had already come to an end. In mid-August, Orlov had been transferred to a labour-camp where he found himself doing heavy physical work at an altitude of more than 1,000 m which left him completely incapable of any serious scientific activity.

By mid-October, when he had moved to another camp, No. 37, his letters once again show interest in scientific matters. One letter deals almost entirely with problems of cybernetics and his concept of "wave logic". There are interesting references to past discussions with Gol'fand, Rozenshtein—presumably within the framework of the refusniks' "Sunday seminars" of which Orlov was one of the few non-Jewish members.

But his conditions of work were still those of hard labour. "I'm working in the plant all week now. I had a choice: cutter or turner. The cutting shop makes a great deal of noise; in the turning shop you have to keep spinning around. I chose the turning shop. I'm still a learner: how it is possible to fulfil one's norm, I still can't imagine. . . ."

Yurii Orlov has seven years to serve in the labour camp, plus five years "internal exile". The avowed purpose of work in a labour camp is rehabilitation through service to the community. In almost every respect, conditions for political prisoners have improved considerably in the 25 years since Stalin's death. The one exception is that of scientists. As Orlov's friend, who forwarded the letters to *Nature* pointed out: "In Stalin's time, scientist-prisoners did research in special prison laboratories — Solzhenitsyn's *First Circle*. They didn't have to waste their talents in physical labour, as Orlov does". ☐

Soviets seek US collaboration over biosatellite

SOVIET space biologists have invited their US opposite numbers to take part in the experimental programme for the next Soviet bio-satellite scheduled for the end of 1979.

This proposed venture was announced by Dr Nikola Gurovskii, a leading member of the Soviet team shortly before a new agreement was signed extending cooperation between the Academies of the two countries. Much of the agreement dealt with details of exchange visits (up to 100 person-months per year) and research visits (16 from each side per year of from 3 to 12 person-month). However, there was also a general acceptance of the desirability of using the new agreement to place greater emphasis on cooperative activities, dealing with important scientific problems—a slot which would neatly take in the bio-satellite.

Dr Gurovskii said that this satellite would include 13 joint experiments. For the first time, the effect of weightlessness on mammals (rats) and birds (quails) would be investigated. There would be further investigations of the physiological effects of space-flight, and also tests on a new "anti-radiation" medicine, using dosimeters manufactured in the US.

These latter aspects are of particular interest to Soviet space medicine, according to a recent article in *Pravda* by Dr Avetik Burnazyan. He stated that the decisive factor limiting space-flights is no longer a technical one, but the ability of the organism to re-adapt to Earth conditions.

The proposed joint biosatellite programme will not be the first time there has been such cooperation; similar though less ambitious joint programmes were carried out on the Kosmos-782 and -936 satellites.

In Dr Gurovskii's opinion, however, the extension of such cooperation is of major importance It is becoming ever more difficult, he said, to develop science in a "narrow national framework". For "truly outstanding results", international cooperation is essential.

Vera Rich

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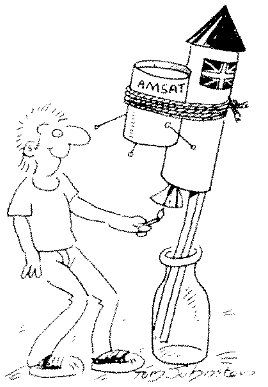
news in brief

Unions refuse to enter plutonium areas at Aldermaston: Management at the UK weapons research plant at Aldermaston has tried unsuccessfully to reopen work areas that have been closed since last August when 12 workers were found with excessive plutonium in their lungs. According to a spokesman from the Ministry of Defence the buildings are open in the active areas but restaffing of the buildings "has not been achieved". The MOD says that the issue under negotiation is danger pay but that it would be "inappropriate to disclose the details". According to the *Observer* (18 February) the unions are demanding £10 per day and management is offering 13p per hr. Bob King, shop steward of the Transport and General Workers Union at Aldermaston, the largest of the four unions involved, says the issue is not danger pay. "We've never said that. It's a term adopted by the newspapers." The TGWU is demanding that the area be made safe before they will allow their members to work in it. The unions will rely on health specialists in their national offices as well as local staff experience to insure that reasonable safety standards are met.

KGB clamps down on Sunday seminar: Reports from Moscow indicate new KGB pressure against the Sunday scientific seminar for refusniks. It is feared that the authorities may be trying to stamp out the seminar altogether—perhaps before the Einstein centenary next month focuses attention on the Jewish contribution to science.

A criminal file, No. 41035/38-75, has been opened on Viktor Brailovskii, the seminar leader, and a certain Avram Yoffe, from Leningrad. One of the people questioned in connection with this file was told by the interrogators that charges were being formulated and a trial could be expected "quite soon". One disturbing fact is that the investigations have been extended to the non-refusniks who have been attending the seminar.

Budget cuts threaten London medical schools: Lord Annan, Vice Chancellor of London University has set up a working party to investigate the financial viability of the University's 12 general medical schools, five dental schools and 13 postgraduate teaching and research institutes. Chaired by Sir Brian Flowers, Rector of Imperial College, the working party is to investigate the effects of further reductions in funds to the university and will prepare plans for the closing of specific schools if it finds this necessary. The action was taken after the Joint Medical Advisory Committee heard numerous complaints that budget cuts were impairing the quality of training. The university trains 30% of British doctors. In 1977/1978 8,250 medical and dental students were enrolled, of which 6,700 were undergraduates and 1,550 were postgraduates.



UK to build amateur spacecraft: Amateur radio enthusiasts at the University of Surrey are to build the UK's first amateur spacecraft—a satellite carrying a series of high frequency beacons which will be used by radio amateurs to study the effects of the ionosphere on radio-wave propagation. It is to be built in close collaboration with the international Amateur Satellite Corporation (AMSAT), the Amateur Satellite Organisation of the UK (AMSAT-UK) and the Radio Society of Great Britain. At an estimated cost of £150,000, the satellite should take two years to build. It is expected to be launched early in 1981 into a polar orbit at a height of 900 km. As all of AMSAT's previous eight amateur satellites have been launched by NASA, it is likely that NASA will be approached to launch this one too. The one exception, however, is the German-built AMSAT satellite which is due for launch by Ariane.

Swiss nuclear safety referendum result: Strict nuclear reactor controls (*Nature* 15 February, page 508) were rejected by the Swiss electorate on 18 February by a vote of 965,271 to 919,217. Nine cantons voted for the referendum and 13 against it. The 49% vote in favour is considered to be a surprisingly good result for the nuclear opposition since the nuclear industry spent 20 million francs on its campaign. So says André Froidevaux, of the National Co-ordinating Committee of Swiss Anti-Nuclear Organisations: "the nuclear lobby successfully exploited working class fears that jobs would be lost if the referendum passed".

An alternative law has been put through parliament. But the anti-nuclear movement has gathered 50,000 signatures to force another national vote on this law on 20 May. The new law gives parliament the right to veto new power stations and requires the need for a station to be documented. There is also a provision giving parliament the right to expropriate land for nuclear waste disposal. This law will be opposed by the National Coordinating Committee on the grounds that it offers virtually no effective controls to reactor development.

Cambridge designers plan low energy homes: A new design of terrace houses that will cut heating costs by 75% from £120/yr to £30/yr has been proposed by the Cambridge based firm, Ambient Energy Design. Their plan, which received first prize at the Energy Show now underway in Birmingham, makes use of better cavity insulation, double glazing units with infrared reflecting film between the panes, roll shades for night time insulation, and a conservatory which will trap solar heat. Gerald Foley of the International Institute for Environment and Development calls the savings "impressive". According to Foley, 30% of primary energy in the UK goes to housing of which two-thirds is for space heating. Thus a cut in heating requirements of 75% means a savings of 15% in the overall energy budget. Members of AED are convinced that low energy consumption represented by the above measures is "economic and realistic and will become commonplace."

Ambient Energy Design—House Heating for the 1980s. 6 Apthorpe Street, Cambridge CB1 5EY, £1.50.

Third World think tank proposed: S. S. Ramphal, Commonwealth Secretary-General has proposed to the South-North Symposium held in Tanzania last December that a Geneva-based research organisation be created to back up a Third World secretariat that can be competitive with existing organisational alliances of the developed world. In 1977 there were over 2,000 meeting days for UNCTAD alone and only 56 of 117 members of the Group of 77 developing countries had missions there. In comparison, organisations like OECD, EEC, and NATO exist to provide cohesion amongst the developed countries. Ramphal proposes that an OECDC, Organisation for Economic Cooperation among Developing Countries, be formed. The OECDC will "of necessity, be technocratic and multinational". Technological improvements in data storage, retrieval and information processing are necessary for the South to negotiate successfully with the North.

US report fails to bridge gap with Third World

The US delegation's paper for the UNCSTD conference in August is a disappointing document, writes **Ward Morehouse**

The long-awaited US National Paper for the UN Conference on Science and Technology for Development has made its appearance more than five months after the UN deadline for submission of national papers. It is strong on diagnosis, but woefully weak on remedies for the economic and social ills of developing countries which are identified in the paper; and unless the US is markedly more forthcoming at Vienna, this document will slip into the oblivion of history as yet another contribution toward the widening gap between profession and performance by industrialised countries toward the Third World.

Let us start with what is right in the report. There is recognition that the transfer of proprietary industrial technology has sometimes had a polarising impact on Third World economies; and that the deals struck by multinational corporations may not have always been fair to developing countries. Indeed the US paper urges that steps be taken to strengthen the negotiating skills of Third World countries on the grounds that more equitable bargains are likely to be more durable.

And the US report rightly recognises the failure of development strategies seeking solely to maximise growth. (Though its assertion that "the 'trickle down' strategy is now dead" taxes the indulgence of many observers.)

There is even recognition of the vital importance of building a strong science base in the Third World and an acknowledgement that most development assistance, focussed on short-term projects with immediate impact, "did not alter developing countries' continuing dependence on the initiatives of the scientific centres of the Northern Hemisphere". In a similar vein the report recognises "the need for developing countries to establish their own policy framework and to set their own priorities", although the report freely indulges in the imperialism of social priorities by repeatedly asserting the primacy in US development assistance of meeting basic human needs.

But the outstanding omission in the report is its failure to reflect the ambivalence of US policy toward the Third World.

In view of the lacklustre and self-serving character of most (but not all) of the other industrialised country national papers for UNCSTD, searching political self-analysis may be too much to expect. But the promising effort to analyse the economic and social shortcomings of US science and technology and other development policies stands in sharp contrast to the lack of political analysis.

While the US and other industrialised countries would no doubt like to see the worst forms of human poverty eradicated and a "self-generating" process of equitable economic growth accelerated in the Third World in the hope of reducing the possibility of overt and violent conflict between North and South, they are also interested in maintaining or extending political and economic dominance in the international system.

This dominance will give them an assurance of continued access to export markets and raw materials in developing countries, thereby helping to retain and enhance their relatively affluent living standards. And as other forms of economic and military power decline, technology is becoming an increasingly attractive and potent instrument for maintaining that dominance. President Carter's National Security Adviser, Zbigniew Brzezinski, has said that "the emergence of more advanced Third World countries like Brazil and Korea as new centres of rapid industrial growth means that the older industrial countries have to rely increasingly on technological innovation to maintain their place in the world."

Therefore it is hardly surprising that the US paper makes no direct reference to Third World efforts to change the rules of the game—for example, the proposed code of conduct on transfer of technology being negotiatied in UNCTAD or the forthcoming conference to consider revision in the Paris Convention on patents.

The report even asserts that "the US Government has been generally neutral toward commercial transactions of US private firms" ignoring the strong political support in international negotiations the government gives to positions which seek to maintain the *status quo* in international technology and investment flows and which serve well the interests of US multinationals Only the scantest reference is given to the powerful influence which US tax, investment, tariff and trade policies have on these flows.

Likewise the report gives little recognition to powerful domestic political and economic interests which shape US development and trade policy toward the Third World—as in the attempt by adversely affected US producers of vegetable oil and other products to prohibit use of US contributions to the World Bank and other multilateral financial institutions for projects designed to increase output of these products in developing countries.

The centrepiece of the US response to these problems is the proposed Foundation for International Technological Cooperation, although it is well known in Washington that the Foundation faces stiff opposition to its creation in Congress and even within the Executive Branch and may never come into being, let alone with the degree of autonomy and magnitude of resources to make a real difference in overcoming the failures of past policy and performance. All in all, it is pretty mild therapy for such a serious set of maladies as identified elsewhere in the US national paper.

The greatest defect of all in the US paper is a failure to recognise that the industrialised countries, and the science and technology on which those countries have a near monopoly, are as much a part of the problem as they are of the solution to the ills of the Third World. The insatiable demand for consumer goods by the rich minority of the world's population has generated technologies which are displacing millions of previously independent farmers and artisans in the Third World from their livelihood.

In the words of the Poona statement calling for open debate at UNCSTD on science, technology and the human condition in the modern world: "They would be players in the theatre of the absurd if they did not occupy positions of power and influence in the real world of today. Humanity must regain its sanity by reasserting the nature of scientific inquiry as the pursuit of truth, leading to technologies which liberate the many rather than facilitate control by the few." ☐

● Last week, President Carter announced the expected reorganisation of the US foreign aid programme. A new International Development Cooperation Administration (IDCA) will administer 2.7 billion dollars of non-military assistance programmes currently controlled by the Agency for International Development. IDCA will include the proposed Foundation for International Technological Cooperation (*Nature*, 25 January) but will be only semi-independent. The State Department and the Treasury will retain ultimate control of any major policy decisions. ☐

Ward Morehouse is president of the Council on International and Public Affairs, and teaches at Columbia University.

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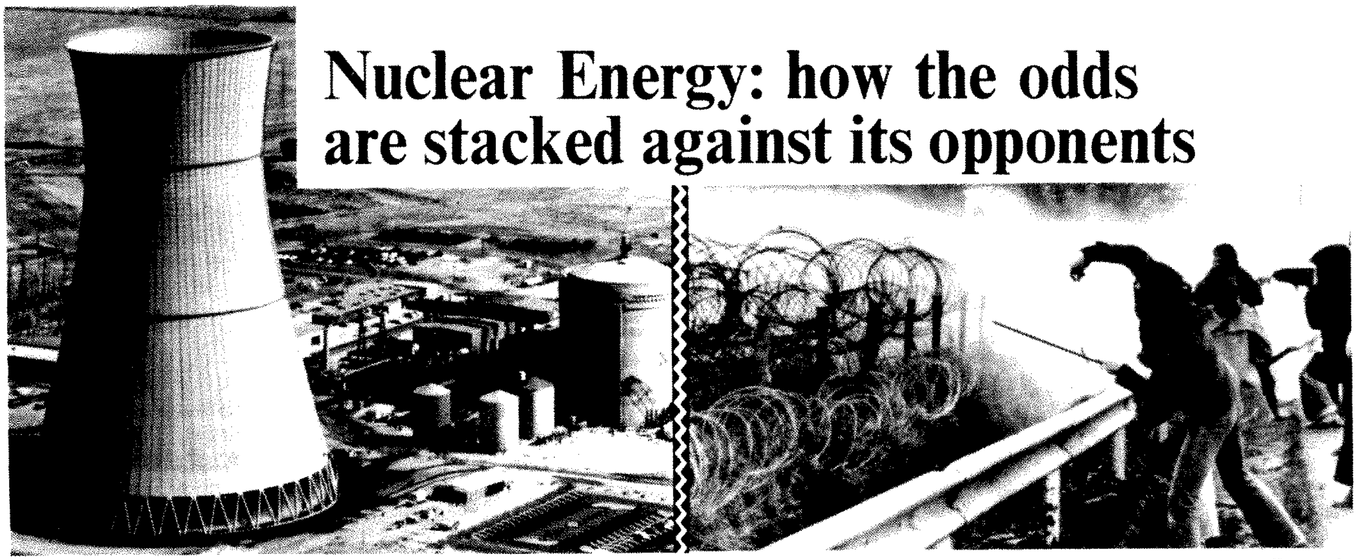
Nuclear Energy: how the odds are stacked against its opponents



● PETER TAYLOR explains why he feels that risk assessment is inadequately incorporated into democratic decision making in the UK.

THE debate over the safety of nuclear reactors illustrates a general problem of dealing with the impact of a technology, namely that the issues are assumed to be primarily 'scientific' and 'technical', and thus to be the exclusive field of 'experts'. Where procedures have been set up to make energy policy decisions or recommendations in the light of this controversy—as at the Windscale public inquiry—expert witnesses have held the arena and a judge appointed to sort out the "facts" and the "truth". At Windscale all witnesses were sworn to "tell the truth, the whole truth, and nothing but the truth". But, as Dr Brian Wynne, who gave evidence at the inquiry, said later: "You can expect a scientist to tell the truth, and nothing but the truth, but not the whole truth."

At the inquiry, Wynne had set out to show how the nuclear controversy differed from true scientific debate. The guarantor of truth in science, he argued, was pluralism in research, availability of information and open critical debate; but the nuclear debate was clouded with secrecy in crucial areas, and critics had totally inadequate resources to match proponents. In safety assessment, for example, there was only one body of facts according to one set of assumptions. Furthermore, scientists within the nuclear institutions, who might wish to dissent, were rigidly controlled by management and by the Official Secrets Act.

The possibility of a major escape of radioactive particles, however much it may now be dubbed incredible by the operators, has influenced siting policy. The early Magnox reactors were all built well away from conurbations. Then, in the late 1960s, pressure mounted to site new reactors near load centres and a review of siting policy took place. The Nuclear Safety Advisory Committee reported that "the major contribution to public safety lies in the standards achieved by design, construction, and operation of the plant". The Ministry of Power then reported to Parliament on 6 February, 1968, that the new advanced gas cooled reactor (AGR) could be operated "nearer to built up areas than we have so far permitted".

At the time of this policy change, the most publicised aspect was the inherently safer design of the AGR, using pre-stressed concrete for the pressure vessel and internal boilers. However, it was also realised that a large release would have a huge impact, however remote the siting. How far this affected siting policy is not clear.

This period of nuclear development was essentially uncontroversial. Assessments of risk were carried out within the nuclear establishment and accepted as adequate by politicians or closed working groups. It was during this period that risk assessment evolved the technique of "probabilistic analysis". Reg Farmer, at the United Kingdom Atomic Energy Authority, developed it as an aid to the designer in deciding what degrees of redundancy and containment might be reasonable to incorporate. He sought target probabilities of failure and the consequences and compared them with other industrial hazards.

The method has its limitations and, to his credit, Farmer has been quick to caution the nuclear industry for taking too much credit for a few hundred reactor-years of safety, and for "failing to see or adequately have regard to all those minor and sometimes major features of equipment or of organisation which might nearly have led to disaster".

Unfortunately this caution has not been repeated by some public advocates of nuclear expansion. The target probabilities of Farmer's curves of frequency against magnitude of effect (in deaths) have acquired a reality comparable with the facts of meteorite impact, and the frequency of dam failures, aircraft crash, and so on. For Sir John Hill, chairman of the UKAEA, such curves "show the probability of . . .", and for Sir St John Elstub (together with various presidents and vice-presidents of certain engineering and chemical institutes in a letter to *The Times*) the fact that Professor Rasmussen "has calculated" the chances of a severe accident for 100 power plants at one incident in 100,000 years is adequate assurance (a conclusion since repudiated by the US Nuclear Regulatory Commission).

The Flowers report served in some way to redress the balance and introduce critical comment into the public arena. The commission noted that in probability analysis and attendant fault-trees not all failures could be foreseen nor all components ascribed a frequency of failure. They concluded: "Whether the safety performance is achieved in practice will depend to some degree on factors assumed in the analysis, such as the quality of maintenance".

Despite this critical attitude to assessment, they were led to conclude that estimates of damage following an accident "must be presented in probabilistic terms". But, in our view, probabilistic presentation obfuscates rather than illustrates the potential effects. A range of incidents under specific conditions of wind and weather, including the worst of such conditions, should be presented. Probabilities are often meaningless: the most that can be said it that the worst case is less likely than the others.

The argument against worst case site-specific studies has always been that when disasters have occurred, the worst has not happened. After the Amoco Cadiz we can expect that this is not an argument that will be repeated for oil slicks. And yet, there is still a

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reluctance to publicise the worst that can happen at Canvey Island or Hinckley Point. We suspect that Justice Parker's attitude prevails: in view of the (accepted) probabilities, such studies would "unnecessarily alarm the public".

That the public might be alarmed is evident when one considers that Flowers' example of a reactor (AGR) accident's consequences, and the NRPB/NII Breeder study, both admit that, under adverse weather conditions, casualties might be ten times worse: in the former case 100,000 casualties; in the latter 600,000.

There is a need for publicly available site-specific studies for AGRs and FBRs (fast breeder reactors) that explore the whole range of potential accidents and which do not slavishly accept the probability calculations of the nuclear establishment. In this, Britain lags far behind the United States and some European neighbours. In the US there have been open critical reviews of risk assessment, aided by the leaking of internal reports, the defection of industry-based scientists, the existence of the Union of Concerned Scientists and the operation of a Freedom of Information Bill. There has thus been an open reassessment of the work of closed expert groups. The most forthright report is that of the American Physical Society's study group on Light Water Reactor safety.

This pattern has been repeated in Sweden, Denmark and Holland, Austria and West Germany, often with the governments funding critical experts. In some cases, dissenting reports have been published by the government printer and incorporated into the procedures leading up to energy policy decisions. Most recently, in West Germany, a panel of 20 leading international, critical experts has been commissioned to review the proposal for reprocessing at Gorleben.

But in the UK, there has been no major reassessment of the work of closed expert groups. Critics are expected to fund their own safety analyses, without research facilities and without access to internal reports on which to base assessments.

Two examples from the work of our group may serve to illustrate the need for independent assessment.

- When the NRPB (National Radiological Protection Board) fast reactor study was published, press comment was largely restricted to assertions of probabilities such as 1 : 1m, and of the worst case being 60,000 casualties. Having been given access, at Justice Parker's instruction, to the UKAEA computer programme, we were able to run the NRPB calculations (which were for a notional site) for a specific locality (we chose Kalkar in West Germany). We discovered that the NRPB had chosen not to present their own worst calculations—the results of one weather category which led to 600,000 casualties. Our site-specific study, having altered only the population data on the programme, produced over 900,000 casualties.

- Hinckley Point has already suffered a potentially severe "loss of coolant" incident. A pipe-break and simultaneous valve failure led to the loss of both primary and secondary cooling. Dr Franklin of the Nuclear Power Company, when asked at Oxford recently about the ascribed probabilities of these events, said he thought cooling had been restored almost immediately through the use of fire-hose fittings. This statement did not accord with that of the Energy Minister in a written parliamentary answer, where we learn that cooling was lost for three hours and that fire-hose fittings were not present on that mark of AGR.

The scale of concern may be judged from the following extract from the proceedings of a Symposium in Vienna in 1967: "Cave and Holmes of the CEGB gave a probability of failure for primary and secondary cooling of 1 : 100,000 per reactor lifetime per independent system. Furthermore they praised the inherent safety of the AGR (over the LWR), in a loss of coolant situation, in that the heat capacity of the boilers, etc., was such that there was a period of three to four hours before the fuel cans would melt."

In the light of this time period, the Energy Minister's comment that there was no danger of a release during the period of loss of cooling takes on a new meaning.

Our concern at this uncritical acceptance of safety assessment has heightened recently with the public debate on the risks of different types of energy production. The Rasmussen-type probabilities have now been factored in to nuclear safety statistics. Thus one severe accident killing, say, 10,000 people in so-many-thousand years is incorporated into a global deaths-per-Gigawatt-year in the same manner as mining deaths are fed into the coal generating option. This initial work by H. Inhaber of the Atomic Energy Board of Canada finds that the nuclear option is safer than either coal, oil or solar. Lord Rothschild used this study in a televised broadcast on risks of energy production.

But Inhaber's work assumes reactor accident frequencies which may or may not be borne out in practice. This has, unfortunately not inhibited its use in the industry's public relations The situation is more disquieting considering that the nuclear establishments have available to them the fruits of an IAEA sponsored research programme on the comparability of risks which clearly highlights the limitations of Inhaber's approach.

The selective use of the results of scientific research, often in an unrefereed form, raises important questions for the future structure of public debate. The problem will arise when considering the forthcoming FBR inquiry. It is our view that AGR and FBR fault analyses will have to be publicly presented and discussed before comparison of energy option risks can be meaningful. If critical experts are to be expected to produce safety analyses, then they will have to have access to official information, if not government support, for their research.

Finally, there is an area of research relevant to safety assessment that is continually neglected, namely the "social facts" relating to the nature of the dispute itself, the role of science, and of controversy. What groups are involved in the debate and what benefits or risks accrue to each (in the nature of contracts, jobs, risk of accident or pollution)? How is risk perceived and evaluated within these groups and what are the components of attitudes? What correlations are there to levels of knowledge of the technology? What procedures exist for debate, participation and information-exchange and how effective have these procedures been in the past?

The controversy itself may bring forth bitter and unresolvable conflict. Once more we find it disquieting that the UKAEA does not choose to publicise the results of further IAEA work in this field. The work of Pahner, Novotny, Stallen and Meertens points clearly to a realisation that the controversy has no resolution that people are divided by a basic evaluatory perception of technology and progress.

We believe that herein lies a fundamental question. The nuclear issue is important as a first testing ground for democratic flexibility. The institutions of industrial growth and technological progress realise that the call by the "alternative" camp for openness, reassessment and participation, however sincere in itself, exposes aspects of their technology which it feels a lay and mistrusting public may not accept. Thus there is a retreat into secrecy and, paradoxically, a massive public information programme. This in itself generates further opposition. We are not convinced that the UK government realises the full impact of this conflict.

Peter Taylor is co-director of the Political Ecology Research Group. This article is based on a talk he gave at a conference on the politics of fast breeders in London at the end of last year The conference proceedings are to be published by Macmillan.

correspondence

The Piltdown hoax: *cui bono?*

SIR,—By far the most sensational feature of the Piltdown hoax, that has remained hidden from the public gaze for so long, is that it was planned and executed by eminent scientists against one of their own number.

Until recently there have been two main hypotheses regarding the identity of the perpetrator of the Piltdown hoax. That espoused by Weiner (*The Piltdown Hoax*, Oxford University Press, 1955), inferring the guilt of the amateur Dawson, is far the more acceptable as it absolves the academic world from any involvement. The other view is that Dawson was the scapegoat, and the hoax was part of a conspiracy on the part of scientists to delude the public into accepting the anti-religious idea of human evolution (M. Bowden, *Ape-men fact or fallacy?* Sovereign Publications, 1977). This thesis, of which there are hints in Langham's letter (18 January, page 170), even suggests that Smith Woodward "may have been a willing accomplice". Hence, Elliott Smith, who nailed his banner firmly to the Piltdown mast, could be imagined to have been the instigator.

Douglas (2 November, page 11) has provided a signal service by making us look afresh at the evidence and consider a "grudge against one of the principals" as a fruitful line of enquiry. The incident of the Sherborne horse's head, if nothing else, was a successful and deliberate demonstration by Sollas of Woodward's incompetence. In this context, it is indeed difficult to imagine Dawson being party to any conspiracy aimed at belittling his close friend of many years standing (from 1884 until his death in 1916). He may possibly have been persuaded to help "encourage the evidence", and it is difficult to avoid postulating some kind of direct involvement. The way in which the desperately wished for lower jaw was flicked up by Dawson's pick, so that it landed literally at Smith Woodward's feet, has such an air of theatre about it that one is bound to be suspicious of such a startling coincidence. When the search was on for the missing canine, Teilhard de Chardin sat down to rest on a heap of gravel that Dawson and Woodward had already sifted. They actually told him that there was no point in searching there, but within a few minutes of casual probing with his fingers the canine appeared, yet another startling coincidence. (This event might well place Teilhard de Chardin among the suspects). The subsequent sudden appearance of the "cricket bat", from the soil under the hedge where they usually lunched, was surely stretching credulity to breaking point. Everything that Smith Woodward wanted, turned up and, moreover, in the order in which he wanted it.

Every good hoax, to be effective, needs to carry certain hallmarks that allow it to be recognised as such by all and sundry, with the main exception of the victim for whom it was designed. Let us now look, not at Woodward's friends, but at his enemies. Douglas has already drawn our attention to the strong possibility of Sollas being involved. Sollas was Britain's leading expert on fossil man, who had just given his Presidential Address to the Geological Society on fossil man and had recently published *Ancient Hunters* (1911). Here was a man who had pioneered over many years the technique of serial sectioning, which enabled palaeontologists to examine the internal structures of fossils that otherwise would never have been accessible for study, a technique which Smith Woodward contemptuously dismissed as a "mere toy". And here was Smith Woodward with pretensions, but no expertise, announcing his ambition to discover the earliest man, and moreover, in Britain. Was this not as close to an invitation as one could imagine?

Weiner (4 January, page 10) draws attention to Oakley's letter to *The Times* (7 November 1978) regarding Sollas's possible use of potassium bichromate, but he seems to have missed my reply (*The Times*, 25 November) refuting Oakley. My letter also mentioned that Dr M A. C. Hinton was the likely source of the medieval orang utan lower jaw and that Hinton had stated that the hoax was initially planned and executed within the Natural History Museum. Unfortunately the short paragraph referring to Hinton's possible involvement was edited out of my original article in *Nature* (2 November, pages 11-13).

In the spring of 1912 Dawson brought the five Piltdown skull fragments to Smith Woodward, but to be certain of its import, it was vital to find the lower jaw. In 1910 Hinton was given the status of a Voluntary Worker in Smith Woodward's Department of Geology to work on fossil rodents. He managed somehow to offend Smith Woodward to such an extent that in 1912 he transferred to the Department of Zoology to continue his work there (eventually in 1936 becoming Keeper of Zoology and retiring in 1945). The available evidence suggests that the medieval orang utan lower jaw came from a box of unregistered material from the Everett Collection to which Hinton then had access. Hinton (*The Times*, 4 December 1953) stressed that the zoologists in the museum would have unhesitatingly referred the lower jaw and canine to a chimpanzee had the material come within the orbit of the Zoology Department! He claimed that in their eyes G. S. Miller's analysis settled the matter and relieved them of the necessity of expressing any opinion and thus arousing hostility within the museum. In retrospect, this epistle from Hinton takes on a new significance.

From the people that knew Hinton well, it is accepted that he must have been involved in the promulgation of the hoax and it is even claimed that he "virtually confessed" to this, prior to his death. Nevertheless, there was always the nagging suspicion that there was a more knowledgeable figure in the background; Douglas's suggestion at last provides the clue to his identity. Douglas asks "Did they [the people who exposed the fraud] ever try to find out if Smith Woodward had any particular enemies who might do such a thing? No. They did not" If a crime is committed against an individual, the first task, having ascertained that the deed has in fact been committed, is to determine if anyone had a strong motive. Such an approach would have pointed in the direction of the Natural History Museum and Oxford. Without wishing to be uncharitable, perhaps this is the reason the grudge theory was never followed up. This would explain the cameo of Sir Gavin de Beer "rolling on his settee, wringing his hands and crying 'What can I do? What can I do?'" as the evidence on Piltdown unfolded. It is not a scandal for an institution to have been duped by a hoax, but if it transpires that it has been initiated in the same institution, it certainly is.

Finally Weiner's accusation of Douglas besmirching Sollas's reputation cannot be left unanswered. Given the circumstances in the 1910-1912 period, given the personality of Smith Woodward, I wonder how many people would have resisted the temptation "to present somebody, who is claiming to be an expert in a field, with something everybody would know is so ludicrous that he would make an utter fool of himself and be shot down in flames". The conspirators misjudged the perspicacity of their colleagues, although Waterston, Lankester, Boule, Miller and Weidenreich doubted the association of ape jaw and human skull from the very beginning. Above all, however, Piltdown engendered such an enthusiastic nationalistic fervour, that the critical faculties of the scientific community seem to have been overwhelmed, and *this* surely was the tragedy of Piltdown.

Yours faithfully,
L. B. HALSTEAD

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False- or pseudogalena

SIR,—Joe Schwartz (25 January, page 255) has confused galena with false-galena or pseudogalena (sphalerite).

Yours faithfully
J. C. KAUER

Du Pont, Wilmington,
Delaware, USA

news and views

Pion interferometry of nuclear collisions

from Miklos Gyulassy

THE measurement of the space-time dimensions of nuclear interaction regions formed during high energy (~1 GeV per nucleon) nuclear collisions obviously calls for rather novel techniques. That interaction region (which we call the nuclear fireball) may be roughly cigar-shaped with longitudinal and transverse dimensions ranging between 10^{-12} and 10^{-13} cm and expanding with relativistic speeds on a time scale of 10^{-23} to 10^{-22} s. In that tiny, short-lived fireball, nuclear matter may be compressed to very high densities (~10^{15} gm cm^{-3}) and heated to enormous temperatures (~10^{12} K).

The physics of nuclear systems under such extreme conditions is now the subject of a new branch of nuclear science known as relativistic nuclear collisions. Recent developments in this area have been reviewed by Goldhaber and Heckman (*A. Rev. Nucl. Part. Sci.* **28**, 161; 1978). One of the most exciting new developments has been the application of pion interferometry to deduce the space-time geometry of that nuclear fireball. The principles behind pion interferometry and the first experimental results are discussed below.

The basic idea goes back to Hanbury-Brown and Twiss (*Nature* **178**, 1046; 1956), who first used the technique of second order intensity interferometry to deduce stellar radii. They made a remarkable observation for the chaotic, incoherent light emitted by stars. While the average intensity (photon count) measured by two detectors A and B was the same, $\langle I_A \rangle = \langle I_B \rangle$, and independent of the separation R_{AB} between the detectors, the average coincidence rate, $\langle I_A I_B \rangle$, did depend on R_{AB}. For large separations $\langle I_A I_B \rangle \approx \langle I_A \rangle \quad \langle I_B \rangle = \langle I_A \rangle^2$, but for small separations, $\langle I_A I_B \rangle \gtrsim \langle I_A \rangle^2$. From the dependence of $\langle I_A I_B \rangle$ on R_{AB}, Hanbury-Brown and Twiss were able to measure stellar radii.

The reason for this variation of the coincidence rate as a function of R_{AB} is now well known and found in any modern quantum optics textbook. It originates from Bose-Einstein statistics required for integral spin particles such as photons. It is the interference between the two parts of the symmetrised wavefunctions, $1/\sqrt{2}\{\psi_1(x)\psi_2(y)+\psi_1(y)\psi_2(x)\}$ describing two identical bosons that leads to the intensity interference, Physically, the enhanced correlation at small R_{AB} follows from the tendency of bosons to congregate in the same state.

Not long after the discovery of the HBT effect in astrophysics, it was rediscovered in high-energy physics in measuring correlations between identical pions. In high-energy physics it is called the GGLP effect (*Phys. Rev.* **120**, 300; 1960). Pions ($\pi^-\pi^-$) are also bosons and are thus subject to the same symmetrisation requirement as are photons. Consequently, intensity interference must also occur for chaotic pion fields. For pions, intensity interferometry involves the comparison of the coincidence rate of identical pions with momenta $\mathbf{k}_1$ and $\mathbf{k}_2$ to the random rate of observing pions with $\mathbf{k}_1$ and $\mathbf{k}_2$. Bose-Einstein statistics lead to an enhancement of the coincidence rate over the random rate for small momentum differences. The correlation function, defined as the ratio of the coincidence to random rate, is thus larger than unity for small $|\mathbf{k}_1-\mathbf{k}_2|$. According to a theoretical analysis by Kopylov and Podgoretski (*Sov. J. Nucl. Phys.* **18**, 336; 1974), the dependence of the correlation function on $\mathbf{k}_1-\mathbf{k}_2$ in fact measures the space-time Fourier transform of the pion source density. The relation between the measured pion correlation function and the source density obtained by Kopylov has been the basis of a great many studies of the space-time dimensions of hadronic interaction regions (see for example Enzell *et al. Phys. Rev. Lett.* **38**, 873; 1977).

Most recently the pion correlation data from high-energy nuclear collisions were analysed by a University of California, Riverside group headed by R. T. Poe. The reaction studied was $Ar + Pb_3O_4 \rightarrow \pi^-\pi^- + X$ using an Ar beam with 1.8 GeV per nucleon kinetic energy (Fung *et al. Phys. Rev. Lett.* **41**, 1592; 1978). In such reactions often more than 100 charged particles (p, π^+, π^-) are observed with negative pion multiplicities up to 15. These are the remnants of the violent explosion of the nuclear fireball. Pion interferometry has given us the first glimpse of the dimension and lifetime of that fireball before its explosion. The group reported that the average radius, R_0, and the lifetime, τ_0, of the fireball were $R_0 = 3.3 \pm 0.9 \times 10^{-13}$ cm and $\tau_0 = 5 \pm (?) \times 10^{-24}$ s.

Although the statistics are as yet too few to determine the longitudinal and transverse dimensions of the fireball separately, the measured R_0 agrees well with our expectations. However, most important, this experiment demonstrated the feasibility of pion interferometry in nuclear collisions. The next generation of experiments with the Bevalac at the Lawrence Berkeley Laboratory are expected to increase the statistics greatly. We will then be able to resolve much more clearly the geometry of these fireballs.

The discussion up to now has been concerned with intensity interferometry when applied to chaotic boson fields. As recently emphasised by Fowler and Weiner (*Phys. Lett.* **70B**, 201; 1977) the intensity interference pattern also depends on the degree of coherence of that boson field. Laser fields, for example, exhibit no intensity interference. The effect of possible coherence on pion interferometry is a topic of current theoretical investigation.

A final comment that must be made is that final state interactions must also be considered in connection with future work on pion interferometry. Unlike photons, pions can interact with each other and the remnants of the exploding fireball. The effects of final state interactions (especially the long-range Coulomb forces) must be unfolded from the measured correlation data before accurate geometrical and dynamical information can be deduced from those correlations. Such studies are also now underway. □

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Exons encode protein functional units

from C. C. F. Blake

An important contribution to relationship between protein structure and the newly discovered mosaic structure of eukaryotic genes, in which coding segments of DNA (exons) alternate with non-coding segments (intervening sequences or introns), is reported by Tonegawa and his colleagues in this week's *Nature* (Sakano *et al.* page 627). The protein chosen was the constant region of the immunoglobulin γ heavy chain. Sequence studies and crystallography have already shown that the constant region is composed of three homologous structural domains: the C_{H1}, C_{H2} and C_{H3} domains. Each domain is composed of about 110 amino acids, folded into almost identical tertiary structures made up from seven β-strands. The C_{H1} domain pairs with the C_L domain of light chains to form, together with paired V_H and V_L domains, the Fab parts of the γ-globulin molecule. The C_{H2} and C_{H3} domains pair with equivalent domains of a second heavy chain to form the F_c fragment, which is separated from the Fab fragment by a hinge region. Each of these structures has a well defined function: the paired C_{H3} domains act in cell surface interactions, the paired C_{H2} domains in complement fixation; the hinge probably transfers information from the Fab to the F_c fragment; the C_{H1} pair is the attachment point for the light chain; the V_H V_L pair forms the antigen binding site; and there is also an N-terminal signal peptide. Thus the immunoglobulin molecule is composed of six discrete structural/functional units whose relationship to the exon/intron mosaic in the coding region could be most illuminating.

The DNA was obtained from mouse MOPC 21 myeloma by complete digestion with *Eco*RI nuclease and the identification of a 6.8 kilobase segment that contained the required sequence by hybridisation with cDNA prepared from MOPC $21_{\gamma 1}$ mRNA. The segment was recombined with the EK2 vector, phage λgt WES, and cloned. After digestion of the clone the position of the γ_1 coding region in the 6.8 kb fragment was determined by electron microscopy of the R-loops formed between it and the γ_1-chain mRNA. This revealed three small R-loops separated by two intervening double-stranded DNA segments, suggesting the presence of three exons interrupted by two introns. A restriction enzyme cleavage map showed that this interpretation was correct, but that there was an additional very short exon located between exons 1 and 2, giving a final pattern of 4 exons separated by 3 introns. Sequence analysis of the intron–exon boundaries and comparison with the known amino acid sequence of the MOPC $21_{\gamma 1}$ chain, revealed that the exons represent, in sequence, the C_{H1} domain, the 14-residue hinge region, the C_{H2} domain, and the C_{H3} domain. There seem to be no introns within the individual sequences coding for these units.

The conclusion from this study, that each of the six known functional units in the γ-globulin heavy chain is coded by a separate exon is most exciting. So clear cut is the correspondence that one is tempted to conclude that the problem of exons has been solved along the lines proposed by Gilbert (*News and Views* **271**, 501; 1978) in which the presence of exons was seen as allowing new proteins to be constructed from parts of old ones. The results reported by Tonegawa are exactly in line with this hypothesis, and show that individual exons can define even very small functional units, like the 14-residue hinge region, and not just domains or folding units as I previously suggested (*News and Views* **273**, 267; 1978). In view of the precise correlation between exons and the functional units in the immunoglobulins, the presence of three exons in the β-globin gene, when the protein cannot readily be divided into individual units of either structural or functional significance seems at first very puzzling. However, closer examination shows that the central exon corresponds very closely to the protein fragment that defines the haem crevice, and contains nearly all the amino acid residues that interact with the haem. Tonegawa's work implies that exons may reveal unsuspected functional protein units—perhaps like the 'minimal globin' outlined above—even when the joins are not visible in the protein structure. The thought that the exon/intron mosaic may carry the frozen evolutionary history of proteins makes one look forward to see whether further determinations of exon–intron patterns of other proteins of known structure support this view, or point in a different direction.

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RNA splicing and polynucleotide evolution

from D. Reanney

Information flow in cells, is defined by the 'central dogma' as

DNA→RNA→Protein

But although the central dogma accurately describes the flow of information in modern cells, it probably fails to reflect evolutionary perspective. Considered from the chronological viewpoint a more accurate representation of the central dogma might be

$$\begin{tikzcd} & \text{DNA} \\ \text{RNA} \arrow[ur] \arrow[dr] & \\ & \text{Protein} \end{tikzcd}$$

This rewriting of a basic tenet of biology has come from several lines of evidence which strongly reinforce the view of Crick (*J. molec. Biol.* **38**, 367; 1968) and others that RNA is the primary genetic material. It is worth re-emphasising that most key features of the translation of genetic information depend on Watson–Crick pairing between (partly) complementary RNAs. Translation is mediated by interactions between different RNAs—mRNA:tRNA; mRNA:rRNA; rRNA:tRNA and rRNA:rRNA. The same was probably true of primitive 'metabolism'. With respect to a metabolic fossil such as the ribodinucleotide NAD, for example, one may ask why is a nucleotide 'handle' a necessary part of a molecule whose active group is nicotinamide. I suggest that the first steps on the road to precellular metabolism were taken when electron-donating and electron-accepting reactions were coupled by means of molecules whose 'handles' were complementary in the Watson–Crick sense. For example

*U*DP-X (red)→UDP-X (ox)
where *U* is paired with *A*
N*A*D (ox)→NADH+H (red)

Here the 'genetic' monomers A and U are used to bring the two elements of the redox reaction into appropriate juxtaposition; this is effectively what an enzyme does. The addition of

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further nucleoide 'handles' would enhance not only the efficiency of the mechanism but also increase its specificity. Reaction specificity is another enzymatic attribute; in terms of this thesis, aminoacyl-tRNAs are but one surviving set of examples of a family of oligonucleotides equipped with non-nucleotide active groups.

Perhaps the most compelling argument for a primary role for RNA in early evolution has come from the discovery of RNA processing. At first sight RNA:RNA splicing seems to remove RNA from early genetic events rather than bring it closer to them. This is because splicing has to date only been demonstrated in eukaryotes. However, the assumption that eukaryotic DNA represents a more advanced type of genetic organisation than bacterial has been suspect for some time (Reanney, *J. theor. Biol.* **48,** 243; 1974). The discovery of split genes has added a powerful buttress to the argument (for a recent, detailed discussion see Darnell *Science* **202,** 1257; 1978).

It is clear that Darnell sees DNA as retaining the genetic function in these early splicing systems. However, the points made by Darnell are consistent with a primary role for RNA both in information storage and information processing. Thus 'mosaic' RNAs produced by splicing are *strictu sensu* recombinant molecules in that they contain data drawn from different parts of the genome. But whereas classical DNA recombination requires the concerted action of a plethora of different proteins (DNA ligases, repair enzymes, denaturation proteins, exonucleases, and so on, splicing requires only two (known) functions—a cleaving and a ligating capacity. Also 'consensus' sequence targets for RNA processing enzymes are short (about six nucleotides (Catterall *et al. Nature* **275,** 510; 1978)) regions on single RNA strands whereas DNA recombination only occurs if two duplex DNAs of strongly matching nucleotide sequence are brought into register. Splicing thus has the virtue of economy which can in this context perhaps be equated with 'simplicity'. It seems logical therefore to suggest that RNA:RNA splicing is the primitive mode of genetic recombination.

One of the better characterised processing enzymes is RNase P. This molecule is a ribonucleoprotein containing about 80% RNA; as such it resembles an undoubtedly primitive structure—the ribosome. Stark *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75,** 3717; 1978) have raised the possibility that RNase P recognises target sequences by RNA: RNA pairing. Since known nucleotide-recognising proteins (such as the *lac* repressor) select their substrates by exploiting the stereospecificity of proteins, the presence of about 350 ribonucleotides in an RNA-cleaving enzyme suggests that the processing of RNA molecules had been fixed into cell chemistry before the takeover of catalytic functions by proteins had been completed. The fact that RNase P processes the products of one of the most ancient surviving genes—that for tRNA—is consistent with this view.

The example of RNase P holds out some tantalising clues as to the origin(s) of the splicing process, conceivably at a time when effectively coded proteins had still to develop. X-ray crystallography has confirmed that the skeletons of 'working' RNAs trace out complex paths through three-dimensional space. Let us assume that RNA: RNA splicing was (and is?) mediated by RNA units which potentiate the cleavage of longer molecules at splice points ('cutter' RNA) and join shorter RNAs ('linker' RNA). (Conceivably both activities could reside in one molecule). A cutter can recognise specific bases in substrate RNA only if these are unpaired; similarly a linker can only act as a ligating agent if bases in the two separate RNAs are unpaired at or near the point(s) where the molecules are to be joined. Since the number and position of appropriately oriented, unpaired nucleotides is determined by the three-dimensional architecture of the RNA it follows that topology must have had a key role in the selection of the splice site—something which seems to survive into the present day since all (known) sequences flanking splice sites are too short *per se* to provide the needed specificity. The (at first sight) puzzling absence of strong internal Watson–Crick pairing at most known splice sites (Catterall *et. al. op. cit.*) is explicable if nucleotides near the site are required to be unpaired in order to provide a recognition mechanism through base-pairing with an independent RNA.

The importance of topology can be illustrated by reference to a specific example of enzymatic interaction with an RNA of known, complex morphology—tRNA. One of the most common modifications to tRNA is methylation, carried out enzymatically using S-adenosyl methionine (SAM) as methyl donor. The function of the various methylase enzymes is to ensure that methyl groups are added at the correct site on the correct base. What is often overlooked is that it is the previous folding of the polynucleotide that determines to a large degree the availability of the bases. So by selectively shuffling base sequences so that sites to be methylated occur in loops it is possible for a low molecular weight agent such as SAM acting at random to achieve relatively specific results. If a useful 'enzymatic' RNA sequence is found among a pool of variants this can be selectively amplified by the autocatalytic ability of duplex RNA; the reproducibility of the modifications (on which such useful enzymatic ability might depend) being ensured by the particular folding mode of transcript RNA.

What are the advantages of RNA: RNA splicing to a very early genetic system? Perhaps the most important is that the 'complexity' of polynucleotides can be increased in a modular fashion as a result of ligation between independently formed oligomers. Further, at a time when the number of effectively self-replicating RNAs was a rate-limiting factor in evolution, processing has the enormous advantage that multiple mosaics can be run off from essentially the same template; new sequences also result from the successive processings of one transcript (the production of multiple mosaics from one RNA was probably inevitable at the time when splicing enzymes had not evolved the precision needed to avoid reading-frame shifts in protein synthesis). Thus processing can maximise the number of sequences exposed to the test of selection in a way and at a rate unachievable through the slow rewriting of existing sequences by genetic noise. The adaptive advantages claimed for splicing (see Gilbert *News and Views* **271,** 501; 1978; Reanney *ICN-UCLA Symp. Persistent Viruses,* 1978) seem to apply with greater force at earlier than at later stages of evolution.

The emphasis placed on 'enzymatic' aspects of RNA implies that, in the absence of sophisticated proteins, the production of mosaics through processing may well have been easier to negotiate than the production of unit mRNAs through punctuation. Excised intronic sequences could readily be used as primers to maintain the continuing replication and transcription of template polynucleotide, thus relegating to RNA yet another role now carried out or aided by proteins.

With the takeover of genetic function by DNA, 'illegitimate' recombination may have complemented the processing mechanism. It is noteworthy that IS*1* and elements of the *att* site of λ contain promotor-like sequences (Landy & Ross *Science* **197,** 1147; 1977). RNA polymerase seems to be directly involved in a *recA*-independent recombination pathway of ρ DNA (Ikeda & Kobayashi *Proc. natn. Acad. Sci. U.S.A.* **74,** 3932; 1977). Illegitimate DNA recombination may thus preserve a memory of the primary role of RNA in early genetic processes.

Since splicing and illegitimate recombination between them provide an enormous pool of 'experimental' polynucleotides one may ask why generalised recombination occurs across the biological spectrum I suggest that this type of recombination developed from the proofreading function which seems to be a universal correlate of DNA synthesis. The proteins needed for generalised recombination could easily have been recruited from the molecules used in the cut and patch mechanism(s) on which proofreading depends One of the most striking things about generalised recombination is its conservative character. No information is gained or lost and gene order is not disturbed Significantly, branch migration and strand isomerisation show up base mismatches as transient heteroduplex lesions. Since these lesions can be recognised and repaired, generalised recombination could be regarded as an extension of the proofreading function from which it evolved. Preferential correction to wild-type rather than mutant genotype can be achieved, at least in phage λ, by enzymatic recognition of base mismatches in non-methylated (that is, newly made) strands (Meselson & Radman cited in *Nature*, **268**, 490; 1977). Thus reciprocal recombination between equal-length DNAs may, in part, be a mechanism for minimising variation, not promoting it.

According to this view the sequence of events in the evolution of genetic recombination was first, RNA:RNA splicing; second, illegitimate recombination and third generalised recombination. Legitimate recombination now acts as a brake on change, continuing evolution being nourished by the earlier mechanisms. □

Short period fluctuations in the Earth's magnetic field

from Ken Games

Up to about 10 years ago, it was generally believed that the magnitude of the Earth's magnetic field varied with periods of approximately 8,000 years on a world wide scale Thus in 1968 Cox (*Science* **163**, 237; 1968) produced a graph of variation in the geomagnetic dipole moment which was obtained by averaging data over 500 year intervals (Fig. 1) In recent years, however, researchers are finding local changes in the magnitude of the magnetic field which have periods of as little as 200 years and which show a change in the total field strength of up to 100%. The questions which now have to be answered are: how confident are we in the magnetic measurements used to determine these changes, and, if these changes are real, what is causing them?

The commonest material used in field magnitude determination is pottery. The main technique used to 'unlock' the magnetic record held in the pottery is that developed by Thellier and Thellier (*Ann. Geophys* **15**, 285, 1959), although many modifications to this technique have since been made. The basic Thellier and Thellier method involves heating a sample to, say, 100 °C in zero magnetic field and allowing it to cool to room temperature The sample is then reheated to 100 °C in a known laboratory field B_{lab} and again cooled. The decrease in magnetic moment m_1 after the first heating represents the magnetisation acquired by the sample in the temperature interval R.T. – 100 °C when the sample was first fired in the ancient magnetic field B_{anc}, and the increase in magnetic moment m_2 after the second heating represents the magnetisation acquired in the laboratory field. Thus the simple equation

$$\frac{m_1}{m_2} = \frac{B_{anc}}{B_{lab}}$$

enables us to determine B_{anc}. This process is then repeated at steps of increasing temperatures such as 200, 300, 400 and 500 °C. If the value of B_{anc} obtained from each step is the same then the result is assumed to be a reliable one. Several possible sources of error in this method have been known and taken into account for a long time. For example, the laboratory heating can cause changes in the magnetic minerals of the sample. This tends to happen at high temperatures, and many of the modifications of the method have attempted to monitor such changes.

Recently, however, two serious possible sources of error have been discovered The first source was reported by Fox, Aitken and Gunn (*Geophys. J. Roy Astron Soc* **53**, 153; 1978) when they discovered a shape anisotropy effect. Thus it was found that the thermoremanent magnetisation (TRM) acquired by a disk-shaped sample (25 mm in diameter and 6 mm thick) along the plane of the disk was about 20% greater than that acquired perpendicular to the plane. This effect seems to be similar to the self-demagnetising effect exhibited by kilns as shown by Aitken and Hawley (*Archaeometry* **13**, 83; 1971)

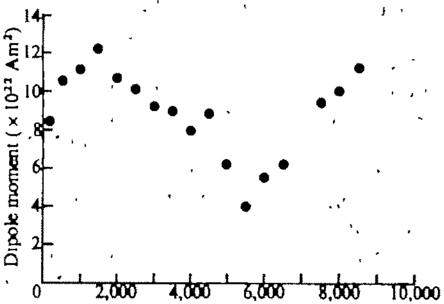

Fig. 1 Variations in geomagnetic dipole moment. Changes during the past 130 yr, as determined from observatory measurements, are shown by the slanting bar at the left. Other values were determined palaeomagnetically (Cox *op. cit.*)

The second source, reported by Rogers, Fox and Aitken (on page 644 of this issue of *Nature*) is another anisotropic problem, this time shown to exist on cylindrical samples 3 mm × 3 mm, which should therefore have no anisotropy due to shape. However, it was found that for samples whose cylindrical axis was perpendicular to the plane of the pottery, the TRM along the axis was up to 40% less than the TRM perpendicular to the axis. This is thought to be due to a preferential alignment of magnetic grains within the fabric of the pottery One would intuitively expect that this effect would be greater for wheel-made pottery than for hand-moulded pottery, but whether this is so or not, it obviously presents a problem in interpreting archaeomagnitudes which have been reported before this discovery. What is certain is that in future care should be taken to give the TRM in the same direction as the normal remanent magnetisation (NRM) was originally acquired, or at least to correct for the anisotropy effect. Even so, there is a further problem yet to be investigated This is the fact that when a sample first acquired its NRM it was part of a larger mass of ceramic, and no one really knows just what difference this makes compared with the acquisition of the TRM after it has been broken away from the larger mass.

One author who has made a correction for the fabric anisotropy is Walton (on page 643 of this issue). His results show a rapid rate of change in the magnitude of the magnetic field in Greece between 2000 BC and AD 400. As stated earlier, these are the sort of results now being found by several workers, and it becomes apparent if the diagram in Fig. 1 is compared with that of Walton, that by averaging over 500-yr intervals Cox has automatically lost the resolution which, given adequate precision of dating, could show the rapid short-period fluctuations now

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being seen. It is also clear that it becomes of vital importance to use material which is very accurately and reliably dated—preferably to within ±30 yr.. Where such dating controls exist, the magnetic results usually show the rapid short period fluctuations.

The major problem still to be answered, however, is what is causing these fluctuations, which can result in a change in magnitude of the magnetic field of up to a factor of 2 in a couple of hundred years. It is difficult to see how the main dipole field can change at such a rate, but before this can be discounted as a possibility we would need to have archaeomagnitudes for the same time period from many different locations, so that an accurate picture of the world-wide rate of change could be produced. The other obvious source of the fluctuations is the non-dipole field. If such non-dipole features did exist at various localities, then as they are so large they must be observable over large areas of the Earth's surface. Hence a study of archaeomagnitudes from areas separated by, say, 1,000 or 2,000 km should enable us to determine how these non-dipole features grew and decayed, and whether or not they showed the Westward drift, which is a feature of the present-day non-dipole field.

As far as the future is concerned, detailed curves of the magnitude of the Earth's magnetic field must be produced for many different areas if we are to understand the reason for the rapid fluctuations now being observed. The material used for this work must be well dated, and as Walton explains, the possibility of then using the curve produced for a given location as a dating tool would be of great help to archaeologists. However, as with all methods of archaeological dating, we must proceede slowly to ensure that the curves we use are as accurate as possible, and that as many sources of error as possible, such as the fabric anisotropy just discovered, are eliminated.

The juxtaglomerular complex: its possible control of multiple nephron functions

from Arthur C. Guyton and John E. Hall

THE discovery of a new secretory cell in the juxtaglomerular complex of the renal nephron is announced in this issue of *Nature* (page 655) by Ryan, Coghlan, and Scoggins. This cell, the granulated peripolar epithelial cell, contains large numbers of granules that are obviously secretory in nature, and it is located in the angle between the glomerular tuft and the outside wall of Bowman's capsule, at a place where its secretion passes directly into the glomerular filtrate as it is first formed at the glomerulus. The authors make the obvious observation that "it is almost inescapable that they [the cells] participate in juxtaglomerular complex [control] activity". But what is the function of these secretory cells?

The juxtaglomerular complex is an anatomical consortium of structures lying at the pole of the glomerulus. It consists of a short portion of the afferent arteriole immediately before it enters the glomerulus and similarly a short portion of the exiting efferent arteriole. And, very important, it also includes a small segment of the distal tubule that crosses the glomerular pole in the angle between the afferent and efferent arterioles and abuts against both these arterioles. This is an invariable structure of each nephron in all higher orders of animals. Obviously, this location of the complex is most propitious for it to act as a controller of nephron function, so much so that essentially every anatomist who has made contributions to its structure has referred to its possible control functions. These include Ruyter (*Ztschr. f. Zellorsch. u. mikr. Anat.* **2**, 242; 1925) who described its general structure, Goormaghtigh (*Comp. Rend. Soc. Biol., Paris* **124**, 293; 1937) who emphasised the importance of 'juxtaglomerular' myoepithelial cells in the arterial walls containing large numbers of secretory granules, McManus (*Q. Jl Micr. Sci.* **88**, 39; 1947) and Schloss (*Acta. Anat., Basel* **1**, 365; 1946) who further defined the interrelationships between the distal tubule and the vascular pole, Hartroft (*Circ. Res.* **12**, 525; 1963) who helped to determine that the secretory granules of the juxtaglomerular cells contain large quantities of renin or prerenin, and many others. In fact Schloss gave the juxtaglomerular complex the name '*der Regulationsapparat*'.

Yet, how much do we know about control of nephron function by the juxtaglomerular complex? The answer is that there has been very little proof of any control function, but on the other hand, large amounts of circumstantial evidence favour several different control functions. In 1964 (Guyton *et al. Circ. Res. (Suppl. 1)* **14**, 1; 1964), we summarised some of this circumstantial evidence and also presented a mathematical model of the manner in which feedback control signals from the distal tubule could 'autoregulate' both nephron blood flow and nephron glomerular filtration rate. Leyssac also suggested a similar mechanism (*Acta physiol. Scand.* **58**, 236; 1963). Since then, many renal physiologists have presented evidence for and against such a method for autoregulation of renal blood flow and glomerular filtration rate. After a long period in which it was disbelieved by most renal physiologists, sentiment seems to be shifting toward acceptance of such a mechanism, so much so that a new term has been coined to describe it, 'tubuloglomerular feedback'. However, there is still much disagreement over what chemical constituent or what physical factor in the distal tubular fluid is sensed to provide the feedback control of nephron blood flow and glomerular filtration.

Nephron function is also controlled by the renin–angiotensin system. At first it was thought that the renin–angiotensin system caused constriction of the afferent arterioles in response to excess sodium or chloride ions in the distal tubules (Thurau & Schnermann *Klin. Wochschr.* **43**, 410; 1965), in this way providing feedback control of the rate of flow of the sodium and chloride ions through the tubular system. However, with the advent of drugs that can block the renin–angiotensin system, we and others have been able to show that autoregulation of renal blood flow becomes better than ever when the renin–angiotensin system is blocked (Hall *et al. Am. J. Physiol.* **232**, F215; 1977). On the other hand, autoregulation of glomerular filtration is better when the renin–angiotensin system is functional. These studies have also allowed us to separate tubuloglomerular feedback into two separate components, one of which controls afferent arteriolar constriction and does not involve the renin system, yet has a strong influence on autoregulation of renal blood flow, but only a weak influence on the autoregulation of glomerular filtration. The second feedback mechanism does involve the renin system which controls the degree of vasoconstriction of the efferent arterioles. That is, when glomerular filtration is too low, feedback from the distal tubule to the juxtaglomerular cells causes renin secretion, formation of angiotensin, constriction of the efferent arteriole, elevation of glomerular pressure, and return of the glomerular filtration rate towards normal.

Thus, the efferent vasoconstrictor mechanism provides an additional quantitative dimension to glomerular filtration autoregulation, adding to the effect also provided by the afferent arteriolar mechanism.

So what control does the secretion from the newly defined peripolar cells exert. As this secretion enters directly

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into the glomerular filtrate, one would suspect control of some aspect of tubular reabsorption. The obvious first question is its possible role in controlling sodium reabsorption, a subject that is still lost in a haze. But we can all think of many other nephron control functions that we would like to see it perform

Thus, the importance of the juxtaglomerular complex grows, not merely for control of a single function of the nephron but perhaps and probably as a controller of multiple functions. □

Trouble with time travel

from Paul Davies

SPECULATION about time travel has long been a source of inspiration to science fiction writers and philosophers, but anathema to physicists. Of course, time travel in a limited sense is known to be an observable fact. Ever since the inception of special relativity, with its celebrated time dilation and 'twins' effects, physicists have recognised that a sufficiently powerful rocket would enable an astronaut to travel into the future. By approaching close to the speed of light, a trip which for the astronaut occupies, say, one year, could return him to Earth to find that fifty years had elapsed there. Indeed, the disparity can theoretically be made as great as one pleases by approaching arbitrarily close to the speed of light The phenomenon is not just a conjecture. Time-travelling subatomic particles are routinely monitored in the laboratory, and the minute, but significant, time dislocations in jet aircraft, have been successfully measured. However, the trip is one way only: there is no going back into the past.

The more entertaining possibility of visiting one's own past is the one that causes physicists headaches, for it plays havoc wtih causality Suppose a time traveller kills his parents before his birth? Even signalling the past can cause horrendous paradoxes. Consider the machine that destroys itself at two o'clock if it receives a message from itself at one o'clock, sent backwards in time from three o'clock. If it blows up at two, no message goes out at three, so no explosion is ordered

It could be argued that a mass of contradictions can be avoided if one only allows a limited range of causally self-consistent loops, thus ruling out 'autocidal' machines and so on *ad hoc*. But there is no doubt that the world would be a safer place if some law of physics could be found that forbids travel into the past The trouble is that, not only do we not know of such a law, we actually do know of some circumstances where our existing theory predicts just such a possibility. The general theory of relativity, which allows space-time to be bent and distorted, describes situations in which world lines (tracks of matter through space-time) are bent right back round and can even 'close up' on themselves, that is, join up with their own past. Thus, a particle can find that its past is also its future—a collapse of causality that few are prepared to take seriously.

One situation in which general relativity seems to predict (if that is the correct word) causality violation is in the vicinity of rapidly rotating massive objects, a celebrated case being the inside of a spinning black hole. Another possibility occurs near a rotating massive cylinder (a more promising case for would-be temponauts). The experts have long regarded mathematical demonstrations of this sort as idealisations, and supposed that in reality some kind of instability would set in to prevent the causality violation occurring There is still a lack of any comprehensive theorems which will demonstrate that general relativity alone saves itself from its own causality-violating solutions, but in 1976, Frank Tipler of the University of California proved that time machines tend to go hand in hand with space-time singularities, also considered to be extremely unpalatable by physicists. So perhaps ridding the theory of singularities would also save causality.

A different possibility has recently been explored by an undergraduate student from Bristol University, now a postgraduate at King's College, London Writing in a recent issue of the *Journal of Physics* (Charlton, *J. Phys.* 11, 2207; 1978) he first investigates the topology of the two-dimensional surface which surrounds the region of spacetime in which particles possess closed timelike world lines, proving it is always a torus. He then goes on to discuss the behaviour of photons close to the time machine region. The result which he finds is that the angular momentum carried by the photon rises without limit as the conditions are approached for the existence of causality violation. In physical terms this means that any loss of radiation from the region will transport vast quantities of angular momentum away from the rotating body. The reaction back on the body itself will cause it to spin down, and hence retreat from the threat of causality violation. Thus, there seems to be an inbuilt mechanism to prevent real objects from rotating fast enough to allow time travel.

Just how general Charlton's mechanism will turn out to be is not yet clear, but it does go some way to confirm what has long been suspected—that travel into the past is purely a fiction. □

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Lunar origin and palaeotides

from David W. Hughes

BEFORE the Apollo missions to the Moon there were three seriously held hypotheses for its origin. The earliest is just over a hundred years old George H. Darwin suggested in 1878 that in early times part of the Earth's crust broke away to produce the Moon, leaving the Pacific Ocean as a scar. This occurred when the Earth had already differentiated into a core and a mantle and was rotating with a period of ~ 4 h. This low spin period caused it to become oblate and unstable More recent additions to this hypothesis suggest that Mars and a myriad of smaller bodies broke away at the same time.

The second hypothesis proposes that the Moon was formed as an independent Solar System planet, further away from the Sun than Earth, and was captured by Earth about 4×10^9 yr ago.

Ring accretion is the basis of the third hypothesis. Earth once had a ring system that may have resembled that of Saturn, the planetesimals within the rings either being derived from Earth by silicate precipitation in a massive high temperature atmosphere or being the remnants of the original condensing planetesimal group that formed the Earth This ring coagulated after it had receded beyond the Roche limit.

Following N. M. Short (*Planetary Geology*, Prentice Hall, 1975) we can list some of the important lunar characteristics that the origin hypotheses have to explain The Moon is very close to being a homogeneous sphere; it has a lower density than Earth, indicating that it has lost iron; there is no water and fewer volatile and siderophile elements than both the Earth and chondritic meteorites, the Moon seems to be enriched in refractory elements, and obviously has a none too primitive composition; it was formed about 4.6×10^9 yr ago, the highland rocks being about 4.3×10^9 to 4.1×10^9 yr old, the mare basalts being about 3.7×10^9 yr old. It seems that the Moon was formed essentially at the same time as Earth and that the majority of the surface evolution took place in the

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first 10^9 years; also there are indications that the Moon was once much closer to the Earth than it is at present.

Unfortunately none of the pre- or post-Apollo discoveries compels the unanimous acceptance or rejection of any of the three hypotheses listed above The authors of these hypotheses, to a man, have found something in the data that apparently supports their point of view. However, A J. Anderson (University of Uppsala, Sweden) is convinced that a detailed study of the figure of the Moon and the tidal forces acting on it in the past (the so-called palaeotides) indicate that the Moon could not have been formed in orbit about the Earth This would automatically rule out the ring accretion and fission hypotheses, leaving only capture. Anderson presents his case in a recent edition of *The Moon and The Planets* (**19**, 409; 1978).

The non-hydrostatic figure of the Moon arose from a distortion introduced by tidal forces some time in the past, a distortion which has been retained by the finite strength of the lunar interior. The gravity field can be charted using Doppler tracking techniques from the Orbiter spacecrafts and the Apollo command service modules that have circled the Moon. This technique led to the discovery of mascons, positive gravity anomalies that existed over certain circular lunar maria basins. These are probably thin concentrations of excess lava that have existed on the Moon for about 3.6×10^9 yr. Isostatic global readjustment has not occurred simply because the viscosity of the lunar lithosphere is of the order of 10^{27}–10^{28} poise, about five orders of magnitude greater than the Earth's

When the maria were formed by the filling of basins with low-viscosity molten lava the resulting maria surfaces would coincide with the gravitational equipotentials A careful analysis of the present-day figure of the maria surfaces gives the remnant static tidal gravity field acting at the time of solidification, and consequently the Earth–Moon distance at that time.

Anderson finds that the older lunar highlands have a smaller tidal ellipticity than the younger circular mascon maria. This is completely contrary to the supposition that the Moon was closer to the Earth when the highlands were formed than it was when the maria were formed. But we know that tidal friction does cause the Moon to recede slowly, in fact the time the Moon spends at a distance R from the Earth is proportional to R^n where n lies between 3.5 and 4.5. Anderson therefore concludes that the Moon was formed away from the Earth and was captured about 3.7×10^9 yr ago just before the time of mascon formation. There is a possibility that the lava flow which produced the mascons was actually caused by the capture. In producing this static tide solution Anderson has to assume spin-orbital coupling. This leads to the further conclusions that the mascon maria flows occurred when the Moon was between 8 and 10 Earth radii away from Earth and that before this the Moon was locked in a configuration with the Earth–Moon line about 15 degrees to the east of its present value. A remnant bulge on the lunar far side, revealed by the Apollo 16 laser altimeter, confirms the fact that just such a reorientation has occurred

So we are left with the lunar capture hypothesis, a hypothesis which unfortunately completely dissociates the origin of the Moon from the origin of the Earth and relegates the cause of their present companionship to a chance encounter during the dawn of the Solar System. If we look at other planetary satellites we can draw comfort from the fact that of the seven lunar-sized satellites, two of them (Moon and Triton) do not have orbital planes which coincide with the equatorial planes of the present planets. Considering all the satellites, about 38% are in non-equatorial orbits, in fact 19% are retrograde. It seems that satellite capture was a fairly common occurrence during the formation of the Solar System. ☐

Pathogenesis of animal trypanosomiasis

from F. E. G. Cox

ANIMAL trypanosomiasis, particularly in cattle, is a major constraint on meat production in Africa. Whether or not the presence of trypanosomiasis, which effectively prevents large scale cattle ranching throughout the tsetse fly belt, is a factor in the preservation of African soil against erosion (Ormerod *Science* **191**, 815; 1976) can be debated but there is little doubt that animals suffering from this disease are not productive and therefore constitute a considerable economic drain on the countries of Africa. Much is known about the symptoms of trypanosomiasis but little is understood of the pathogenesis of the disease and a recent meeting* brought together a number of scientists working on this and related problems.

The most important species of trypanosome causing animal trypanosomiasis are *Trypanosoma vivax*, *T. congolense* and *T. brucei* which infect cattle, sheep and goats as well as wild and laboratory animals. The pathogenic effects vary from parasite to parasite, host to host and even individual to individual. This is partly because the trypanosomes occupy different sites in the body, *T. congolense* and *T. vivax* are normally confined to the blood while *T brucei* is found in both blood and tissues, and partly because pathogenic changes may be caused by the trypanosomes themselves or by the hosts' efforts to rid themselves of their infections All three species undergo antigenic variation so in most animals there is little or no protective immunity while ineffective immune responses bring about a number of deleterious immunopathological changes.

M. Murray (ILRAD, Nairobi) suggests that the pathogenic changes during trypanosomiasis in cattle proceed in three phases; anaemia with high parasitaemia and enlarged spleen, anaemia with transient parasitaemia and variable spleen size and, in those animals that recover, anaemia without parasitaemia and the spleen returning to normal. However, dramatic changes occur within a matter of hours and many cattle die without showing major signs of disease (G. Losos, Veterinary Research Department, Muguga, Kenya) The inadequacy of the immune response is indicated by the fact that animals challenged a year after recovery usually go through the whole cycle again During infection, irreversible lesions occur and complement levels, for example, never return to normal (H. Tabel, University of Saskatchewan).

There are probably several causes of the characteristic anaemia of trypanosomiasis. There is little doubt that a haemolytic factor is involved and one possible candidate is a phospholipase associated with dying or dead trypanosomes (I. Tizard *et al.* University of Guelph). This is the first defined toxic product of a trypanosome to be implicated in the pathogenesis of trypanosomiasis and the long search for such substances, begun in the time of Landsteiner, is obviously well worth continuing There is also evidence that

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*A Conference on Recent Advances in the Knowledge of Pathogenicity of Trypanosomes sponsored by the Canadian International Development Agency (CIDA) and International Development Research Centre (IDRC) and the International Laboratory for Research on Animal Diseases (ICRAD) was hosted by the Veterinary Research Department of the Kenya Agricultural Research Institute, Muguga and was held in Nairobi, Kenya, on 20–23 November, 1978

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immunological responses initiated by the trypanosomes themselves are involved in red cell lysis (Tabel)

Following the initial infection, pathogenic changes occur so rapidly that it is almost impossible to unravel those that result directly from the presence of the parasites from those resulting from the hosts' attempts to compensate for damage caused by anaemia and immunological responses to parasite and possibly host antigens. Nevertheless, the disease has many of the characteristics of an inflammatory one and P F L Boreham (Imperial College, London) postulates that antibody–antigen complexes initiate a series of reactions leading to the activation of kallikrein and eventually increased capillary permeability on one hand and disseminated intravascular coagulation on the other These effects, and the anaemia, are only part of the whole pattern of pathogenesis and virtually every kind of cell (M G. Maxie & V E O. Valli, University of Guelph), tissue (W I. Morrison & M Murray, ILRAD, Nairobi) and organ (Valli) may be involved. The dynamics of the various changes can now be determined very accurately using a number of tracer and radioactive labelling techniques (J. D Dargie, FAO/IAEA, Vienna) and these techniques will become increasingly useful in the next stage of unravelling the various intertwined reactions.

No conference on trypanosomiasis could be complete without some consideration of the ways in which meat-producing animals can be kept in regions of endemic trypanosomiasis. The possibility of using cattle tolerant to trypanosomiasis is not an immediate one as carefully controlled studies have now shown that the reputed trypanotolerant breeds like the N'dama do suffer considerably from trypanosomiasis although not as much as Zebu (Murray, Dargie) but much more quantitative work is required on this subject. On the other hand, young cattle do not seem to suffer so severely from trypanosomiasis as do adults (B T. Wellde, Walter Reed Institute Washington) and eland that can be bred in captivity do seem to recover from infection (R Olubayo. Veterinary Research Laboratory, Kabete, Kenya). Perhaps we should acknowledge the supremacy of the tsetse fly at least temporarily and turn our attention to less popular breeds of cattle and alternative meat-producing animals which will eventually be able to live in Africa together with the tsetse fly and the trypanosomes but in the absence of clinical trypanosomiasis. ☐

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Pacific quiet zone contracted

from Peter J. Smith

LARGE-amplitude linear magnetic anomalies have now been used to interpret vast areas of the Earth's ocean floor in terms of plate tectonic processes But there are still gaps; there are still difficulties in some areas, not least in the western Pacific. The late Mesozoic pattern of magnetic lineations in this region was first unravelled by Larson and Chace (*Geol. Soc Am. Bull.*, **83**, 3627; 1972) who traced seafloor spreading back to the early Cretaceous and later to 153 million years (see, for example, Larson & Hilde *J. geophys Res.*, **80**, 2586; 1975). But beyond that, in both temporal and geographical senses, there is a large zone of low-amplitude $(<100\gamma)$ anomalies, known as the Pacific Jurassic quiet zone (PJQZ), surrounding the Mariana Basin.

Whatever the cause of the PJQZ, its existence has impeded the resolution of the area's tectonic history and may have led to unnecessarily forced interpretations. For example, Hilde *et al* (*Tectonophys.*, **38**, 145; 1977) suggested that the Mariana Basin may be underlain by Cretaceous crust formed during the better-documented Cretaceous magnetically quiet period—a proposal which implies, of course, that the basin originated during a time of intraplate spreading about 100 million years ago In other words, by this interpretation, the magnetic anomalies in the western Pacific get older and older until they reach the PJQZ boundary at 153 million years, beyond which the lithosphere is younger

This is possible, but unlikely. Moreover, as Cande *et al.* now suggest (*Earth planet. Sci Lett.* **41**, 434; 1978), such contorted theorising is unnecessary. Cande and his colleagues show (from existing data) that in the PJQZ, immediately beyond the 153 million year line, the magnetic anomalies are apparently no different in character and lineation, but differ only in amplitude, from those on the younger side of the line. It is necessary to say 'apparently' here because it is just possible that the older alternating positive and negative anomalies are not the result of field reversal at all but of field magnitude fluctuations within a single polarity; and certainly the observed anomalies may be modelled successfully on either assumption. But just as there is no doubt that the anomalies younger than 153 million years are produced by a combination of seafloor spreading and geomagnetic field reversal, there is little reason to suspect that the older anomalies are not produced likewise Cande *et al.* have thus been able to extend the Mesozoic polarity-time scale, temporally, about 5 million years beyond the previous limit of 153 million years and geographically closer in towards the Mariana Basin from three different directions.

On this basis the field reversal rate during those 5 million years within the PJQZ was calculated to be once about every 100,000 years. But if the 153 million year boundary has no significance in terms of field reversal, seafloor spreading and the shape of the resultant magnetic anomalies, why is there a PJQZ at all? The first thing to be said about this is that the PJQZ boundary is not as sharp as it is sometimes imagined; there is no sudden change in anomaly amplitude at 153 million years. In fact, the amplitude begins to decrease somewhere around 140 million years and continues to do so fairly smoothly up to 155–160 million years. Or to rephrase this in forward time terms, from 160 to 140 million years ago the anomaly amplitude in the western Pacific increased fairly smoothly, actually by a factor of about four

This increase in amplitude could have been due to a real increase in the magnitude of the geomagnetic dipole moment. Alternatively, it could have been the result of a gradual decrease in the frequency of (undetected) short polarity events, the presence of which would give an apparent reduction in the amplitudes of the observed anomalies. On the basis of the small-scale magnetic detail observed in the profiles from the faster-spreading sections of the western Pacific, Cande and his colleagues conclude that the first of these explanations is the most likely Either way, the field of uncertainty in the western Pacific has been reduced, and in such a way as to suggest that further reduction may soon be possible ☐

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A hundred years ago

AT the last meeting of the French Geographical Society a letter was read from the Abbé Desgodins, dated Yerkalo, August 27, 1878, in which he states that, contrary to the common assertion which represents the sheep as the beast of burden most used in Thibet, this function belongs in preference to the yak (*Bos grussiens*); the the mule, ass, and horse are also made use of.

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review article

Laser isotope separation

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It is about 10 years since new methods of isotope separation by means of laser radiation were first proposed. In the past five years a lot of effort has been put into elaborating and realising these techniques. Now we are at the threshold of demonstrating the first pilot set-ups of laser isotope separation (LIS). In this review I will try to outline the basic ideas, methods and key experiments.

THE successful elaboration of LIS methods at the beginning of the 1970s became possible only because of the development of a variety of tunable lasers covering a wide spectral range from about 2,000 Å to 20 μm. Moreover, LIS methods pose stringent requirements for the parameters of tunable lasers and thus stimulate additional research into the development of highly efficient tunable lasers. (For more detailed reviews see refs 1-5.)

Ideas and various LIS methods

All LIS methods are based on a series of consecutive processes which start with the selective photo-excitation of an atom or a molecule of the chosen isotopic composition. Isotopically selective photoexcitation is possible because of the difference of frequencies and structure of spectral absorption lines of atoms and molecules of different isotopic composition[6,7]. Isotopic effects appear in optical atomic spectra due to the difference of masses, volumes and nuclear spins of different isotopes[8,9]. The difference in isotope mass which affects the frequency of vibrations and rotations of a molecule is essential for molecular spectra[10–12]. Spectral lines in a gas of low pressure (less than a few torr) are very narrow, so that a small isotopic shift is sufficient for selective excitation of the chosen isotope. However, there are some important exceptions (atomic spectra of alkaline and alkaline-earth elements, spectra of polyatomic molecules with heavy isotopes at room temperature) for which special methods of narrowing of spectral lines (atomic beams[13]) and of simplification of complex molecular spectra (cooling of molecules in a gas or in a supersonic jet[14–16]) are applied. Broadened spectral lines of molecules in a gas at a pressure of more than a few torr and in a condensed medium are not appropriate with rare exceptions (low temperature matrices[17]) for isotopically selective excitation.

The excitation of an atom (molecule) changes some of its physical and chemical properties (Fig. 1) noticeably. First, the excited atom (molecule) usually has a higher chemical reactivity than the unexcited one, as the acquired internal energy increases the probability of overcoming the energy barrier of a chemical reaction. The photochemical approach to isotope separation is based on the ability of selectively photoexcited atoms (molecules) to react chemically with a suitable partner at a rate considerably exceeding the rate of chemical reaction of unexcited atoms (molecules) of another isotopic composition. This approach is well known and was successfully used in the photoexcitation of electronic states of some atoms and molecules before lasers were invented[18–24].

Second, the excitation of an atom markedly decreases its ionisation energy and hence selectively excited atoms can be ionised by additional radiation of a suitable wavelength, leaving unexcited atoms of different isotopic composition untouched. This idea forms the basis of the atomic photoionisation approach to LIS.[25–27]

Third, the excitation of a molecule reduces its dissociation energy quite significantly and hence selectively excited molecules can be dissociated much more easily than unexcited molecules of another isotopic species. This mechanism is the basis of the first photodissociation methods of LIS developed[27–29].

These three main ideas are the basis of the fundamental actively developed trends (photochemistry, photoionisation and photodissociation) in LIS considered here. Several other less universal, but also interesting, methods of LIS use photopredissociation[30,31] and photoisomerisation[32] of some molecules, and photodeflection of the atomic trajectory at photon absorption due to the recoil effect[33]; other methods are also proposed and demonstrated.

Photoionisation methods

Selective photoionisation of atoms is the most universal photophysical method for selective separation of substances, particularly isotopes, at the atomic level. A feature common to all

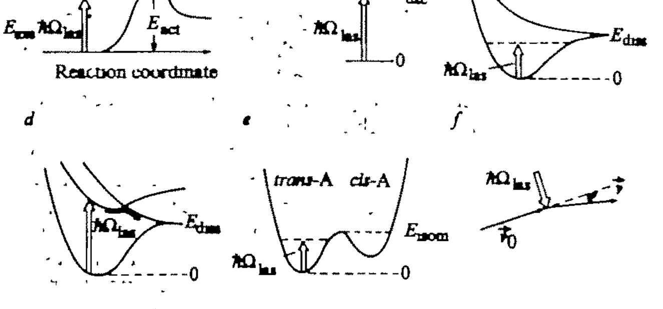

Fig. 1 Atomic and molecular properties which vary during laser excitation: (a) enhancement of reactivity; (*b*) reduction in ionisation energy; (*c*) reduction in dissociation energy; (*d*) photopredissociation((*e*) photoisomerisation; (*f*) change in mechanical trajectory.

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schemes for selective ionisation, is the sequence of the two steps: (1) isotopically selective excitation and (2) ionisation of the excited atoms. Figure 2 shows the main schemes of selective atomic ionisation of special interest for LIS. The two-step photoionisation scheme[23–27] is the simplest. The three-step scheme may be useful for ionisation of atoms with a high ionisation potential using visible lasers. The photoionisation cross-section can be increased by tuning the frequency of the last-step radiation to that of a transition to an autoionisation state: either a spontaneous[34] or an electric field-induced[35] state. Finally, high Rydberg states can be ionised by IR radiation[36] or pulsed electric fields[35]. All these schemes have already been used for isotopically selective ionisation. Their advantages and disadvantages are not discussed here but detailed information is given in ref. 37.

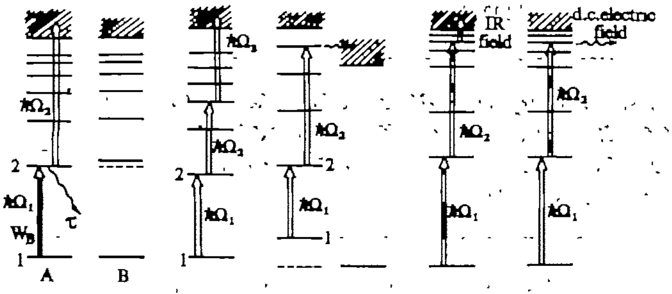

Fig. 2 Mechanisms of isotopically selective step-wise photoionisations of atoms by laser radiation; *a*, two-step photoionisation, *b*, three-step photoionisation; *c*, stepwise photoionisation through an autoionisation state; *d*, selective excitation of a Rydberg state and its photoionisation by IR laser radiation; *e*, selective excitation of a Rydberg state and its ionisation by electric field.

A great deal of attention has been paid to uranium isotope separation (some research programmes are described in refs 38–42). At low density of uranium vapour in an atomic beam (10^{10} cm^{-3}), a 70% isotope enrichment of ^{235}U uranium ions has been obtained as a result of selective two- or three-step ionisation of ^{235}U atoms by dye laser radiation. As the abundance of ^{235}U in natural uranium is only about 0.7%, such an enrichment corresponds to an isotope separation factor of about 100. The operating conditions of the LIS set-up at such low vapour density in the atomic beam are far from optimal and in order to obtain the necessary production capacity and high efficiency of the applied laser radiation the size of the equipment should be very large. Similar experiments at higher density of uranium vapour (10^{13} atoms cm^{-3}) have yielded 6% enriched ^{235}U (ref. 42). This indicates that this process is well adapted to produce light-water reactor fuel but less suitable for highly enriched uranium. The considerable technical problems of such a method of uranium LIS, for instance, the high chemical reactivity of uranium vapour at the high temperatures necessary to produce the required density should also be kept in mind. Therefore there are some problems in industrialising the atomic ionisation process of uranium LIS. But the suggestion that such a process will lead to the cheap production of highly enriched uranium in moderate-size laboratories, is somewhat exaggerated[43].

Besides the uranium isotope separation, which demands rather different economic technological conditions, laboratory experiments are being carried out on the selective ionisation of other isotopes, which are potentially useful in much smaller quantities, such as Ca (ref. 13), Rb (ref. 44), rare-earth[45], and probably transuranium elements. Effective ionisation of excited atoms at a moderate average intensity of the ionising laser radiation, on laboratory LIS set-ups presents a major problem in these cases. The ionisation schemes given in Fig. 2*a,b* cannot be practically applied here, and so greater attention must be paid to the ionisation scheme of highly excited atoms shown in Fig. 2*d,c*. The ionisation of high-lying states by an electrical field pulse[35,44,46] is the most simple and universally applied method. This method of stepwise ionisation has been reported in detail elsewhere[46–50] for various atoms.

The discovery of a long-lived (~1 ns) autoionisation state in the spectrum of Gd atoms[51] with its large transition cross-section of 10^{-15} cm^2 shows that such a scheme can sometimes compete with the scheme which uses the electrical pulse for ionisation of a highly excited atom.

The method of multi-step selective ionisation of atoms through the high-lying sharp quantum states in the vicinity (below or sometimes above) of the ionisation limit is quite suitable for LIS, at least on a laboratory scale.

Photodissociation methods

Various schemes of isotopically selective photodissociation of molecules through the excited and/or the ground electronic state are shown in Fig. 3. These were first proposed, experimentally realised and used for isotope separation at the Institute of Spectroscopy of the Academy of Sciences of the USSR. They are now widely used both for LIS and for potential applications in photochemistry, phototechnology of materials, and most recently, in photobiology.

All these schemes are based on primary isotopically selective excitation of vibrational states of molecules by IR laser radiation as long as the vibrational spectra have considerable isotopic shifts (especially for light elements). In the simplest case (Fig. 3*a*) the molecules excited to the first vibrational level can be dissociated by irradiation of an appropriate wavelength[28,29] following the excitation of the unstable electronic state. The first successful experiments on isotope separation using two-step (IR+UV) molecular photodissociation were carried out on molecules of ^{14}NH$_3$ and ^{15}NH$_3$ (refs 29, 52) and the corresponding experiments on molecules of ^{10}BCl$_3$ and ^{11}BCl$_3$ were carried out[53] later.

The disadvantage of such a method is the comparatively small redshift of the UV photodissociation absorption band after excitation of a molecule to the first vibrational level. This limits the isotopic selectivity of IR+UV dissociation. This disadvantage disappears when exciting higher vibrational levels due to the multi-step or multi-photon process (Fig. 3*b*). This has recently been shown in experiments[54] with ^{12}CF$_3$I and ^{13}CF$_3$I molecules using an IR CO$_2$ laser and UV excimer laser, where the dissociation selectivity has been found to be >100.

An impressive and productive discovery was the isotopically selective photodissociation of polyatomic molecules BCl$_3$[55], SF$_6$[56]) by intense IR CO$_2$ laser radiation. The discovery of this effect was preceded by studies[57–61] of the interaction of intense IR CO$_2$ laser pulses with molecular gases absorbing in the region of 10 μm. It was found that under relatively intense IR radiation during a short time (10^{-7}–10^{-9} s), a polyatomic molecule could pass through the vibrational levels reaching even the dissociation limit (Fig. 3*c*), without colliding with other molecules. The whole process of multiple absorption of IR photons begins

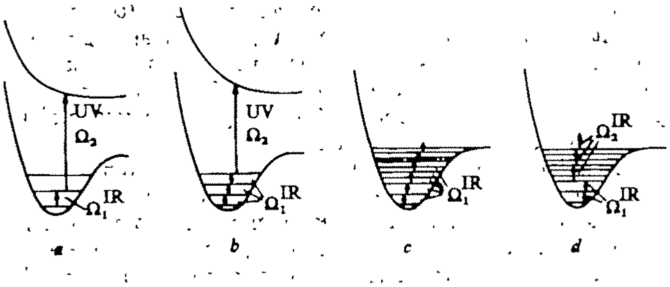

Fig. 3 Schemes of isotopically selective multi-step photodissociation of molecules by laser radiation through an excited electronic state (*a*, *b*) and the electronic ground state (*c*, *d*): *a*, two-step IR+UV photodissociation; *b*, multi-selective excitation of high vibrational levels and their photodissociation by UV radiation; *c*, multiple photon selective excitation and dissociation by a single frequency intense IR field; *d*, multiple photon selective excitation of vibrational levels by resonant IR field and their multi-photon dissociation by non-resonant intense IR field.

with a low vibrational transition where there is usually a marked isotopic shift (at least for isotopes of light and moderate masses). By choosing an appropriate IR-radiation frequency the dissociation of some isotopic molecules can be achieved. The case of isotopic SF_6 molecules is shown in Fig. 4. When the frequency of powerful pulsed CO_2 laser radiation with pulse energy of several J cm^{-2} is tuned to the absorption frequency of the chosen isotopic molecule, preferential photodecomposition takes place. This is demonstrated by the change of the isotopic composition (enrichment) of the undissociated SF_6 molecules and of products formed as a result of dissociation.

Much information about isotope separation by dissociation has been obtained using multiple photon absorption of IR radiation for a large number of polyatomic molecules, including BCl_3 (refs 55, 62, 63), SF_6 (refs 56, 64, 65), OsO_4 (ref. 66), CCl_4 (ref. 67), SiF_4 (ref. 63), $C_2F_2Cl_2$ (ref. 63), CH_3NO_2 (ref. 68), MoF_6 (ref. 69), $H_2Cl_2C_2$ (ref. 70), H_2CO (ref. 71), SeF_6 (ref. 72), CF_3I (refs 73, 74). The dissociation of SF_6 molecules, in particular, has been widely investigated. A detailed description of the method of multi-photon IR laser photochemistry is given in refs 4, 5, 75.

The abundance of vibrational–rotational transitions in a polyatomic molecule (which increases sharply after absorption of several IR photons) is the main reason for this ready absorption of many photons up to the dissociation limit. There is no such multiple absorption of IR photons in simple (two- or three-atomic) molecules where the number of possible vibrational–rotational transitions is limited. An excited polyatomic molecule absorbs IR photons by many different vibrations which leads to a strong excitation of a large number of (perhaps all) vibrational degrees of freedom. In this case the high vibrational temperature can be assigned to the excited molecules (thermal model[75,76]), and its dissociation can be described by the well known theories of monomolecular reactions[77].

An important modification of this method is the scheme shown in Fig. 3*d*, when dissociation is carried out by two pulses of IR radiation at different frequencies[78]. In this way it is possible to separate the functions of isotopically-selective excitation of a molecule and the dissociation of selectively excited molecules. For selective excitation of a molecule, radiation of a finely tuned frequency ω_1 and intensity is required. Dissociation of selectively excited molecules requires intense radiation of only roughly controllable frequency ω_2. In this case, isotopic selectivity of dissociation is enhanced as strong non-resonant radiation at frequency ω_2 does not perturb the resonant transition at frequency ω_1. This method is particularly important for isotope separation of heavy elements. Recent experiments[79] on OsO_4 molecules have shown that it is possible to obtain a separation selectivity of about 1.6 using two-frequency dissociation, provided all the parameters are chosen correctly; for one-frequency dissociation, however, when a natural mixture of isotopic molecules is irradiated, no isotopic selectivity is obtained.

Recent experiments[80,81] on multi-photon dissociation of UF_6 molecules by 16 μm radiation pulses of a CF_4-laser, that is at the frequency of the vibrational band ν_3 of UF_6, were carried out according to the simplest scheme of dissociation in a single frequency IR field (Fig. 3*c*). Dissociation characteristics (threshold energy, yield) resemble in many respects the corresponding characteristics for SF_6. The next obvious step is to obtain the required dissociation selectivity (about 4) using the more delicate schemes of dissociation shown in Fig. 3*b,d*. A more reliable way of increasing the UF_6 dissociation selectivity is to use dynamically cooled UF_6 to provide distinct resolution of vibrational–rotational absorption lines of $^{235}UF_6$ and $^{238}UF_6$ (ref. 83).

Photochemical methods

So far, the possibilities of photochemical isotope separation have been shown by using the excitation of atomic and molecular electronic states and of molecular vibrational levels. However, in spite of the optimism of the early research, the actual advances made seem much less impressive than for the photophysical (photoionisation and photodissociation) methods discussed above.

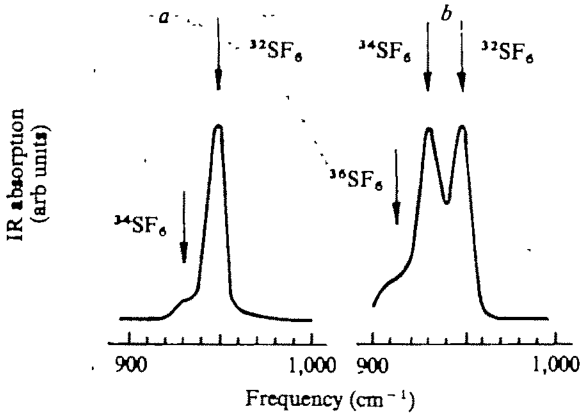

Fig. 4 The infrared spectra of SF_6 before (*a*) and after (*b*) irradiation by focused pulses of TEA CO_2 laser. The number of laser shots was chosen so as to equalise the concentrations of $^{34}SF_6$ and $^{36}SF_6$ (ref. 56).

The chemical reactions of electronically excited atoms and molecules have been studied for many years—especially before the application of lasers. The photochemical isotope separation of Hg excited by the 253.7-nm resonance line has been successfully demonstrated with a variety of reagents[18–24]. For example, Pertel and Gunning[23] were able to enrich ^{202}Hg from 30% of its natural abundance to 85%. The kinetics of the photochemical reactions of Hg are so complex that even the most extensive work does not yield a complete mechanism. Successful experiments on isotopic separation of nitrogen[84] (or resonance lamp for excitation of NO) and carbon[85] (iodine resonance lamp for excitation of CO) have subsequently been carried out. An enrichment factor of ~4–6 has been achieved.

Laser sources for photochemical isotope separation have many obvious advantages over incoherent sources. The broad tunability and high ultimate resolution of lasers give a comparatively free choice of absorption lines, while permitting the highest possible selectivity. This makes the process of photochemical separation of isotopes much easier. It is basically impossible to carry out photophysical experiments of LIS with the usual noncoherent sources of light, because their low radiation temperature means they cannot sufficiently populate the intermediate quantum states and hence cannot provide the effective multi-step process of excitation.

Several successful experiments on laser photochemical enrichment have been reported. The most interesting results have been obtained in experiments on photochemical separation of ^{35}Cl and ^{37}Cl through isotopically selective electron excitation of ICl molecules by cw dye laser radiation[86–88]. For example, in the case of selective photo-addition of ICl to acetylene, an enrichment factor of ^{37}Cl of 100 was obtained[88], in the photoproduct C_2H_2ICl. Both stable chlorine isotopes can be enriched to more than 97% purity in a single step, starting from natural samples. This is a most promising result in isotopically selective electronic photochemistry.

The rate of a chemical reaction may be substantially enhanced by vibrational excitation of the reactant molecules, and it was suggested as a method for LIS in 1963 (ref. 89). However, the first successful experiment was not until 10 years later[90] when ^{35}Cl was enriched by enhancing the reaction of Br atoms with vibrationally excited $H^{35}Cl$. An HCl-pulsed chemical laser was used to excite HCl from $\nu = 0$ to $\nu = 1$ and to $\nu = 2$. For equal pressures of Br and HCl a separation factor of 2 was observed. Recently, carbon isotopic enrichment was also observed in the reaction of vibrationally excited CH_3F molecules with bromine atoms[91]. Although the observed enrichment factor was small, this experiment is interesting from the point of view of using a cw IR laser for LIS.

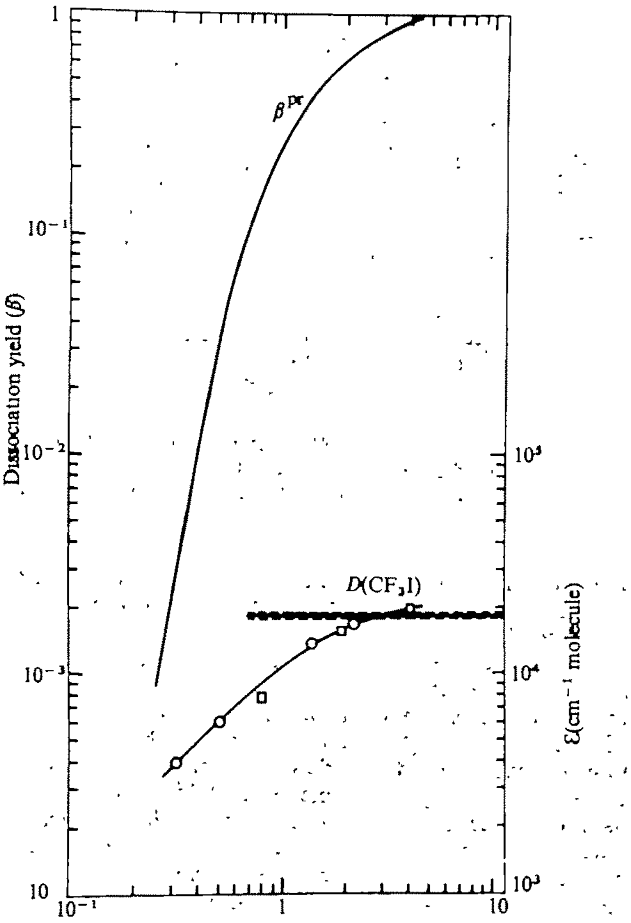

Fig. 5 The dependence of the average energy $\bar{\varepsilon}$ absorbed by CF_3I molecule (curve 1, right scale) and of the primary dissociation yield at 0.2 torr CF_3I (curve 2, left scale) on the energy of the CO_2 laser pulse[74].

Pumping of BCl_3 molecules in a mixture of BCl_3+H_2S (or D_2S) by a focused, pulsed CO_2 laser, which probably excites high vibrational levels, caused separation of boron isotopes[92]. Clearly this experiment is intermediate between single-photon IR photochemistry in a weak field[89–91] and multiple photon IR photochemistry in an intense field[55,56].

Potential advantages of LIS

The laser method of isotope separation has several potential advantages over the more traditional methods (see ref. 93).

(1) High selectivity of elementary processes of separation. The separation selectivity on one stage α in most traditional methods is very small: $\alpha - 1 \ll 1$. Therefore, many stages of separation N are needed to obtain a high enrichment factor $K = \alpha^N \gg 1$. Laser methods provide $\alpha \gg 1$, which reduces the number of separation stages in most cases to only one or two.

(2) Possibility of extracting the desired isotope leaving other isotopes untouched. When the element has a few isotopes with intermediate masses, we can extract only the desired isotope using a tunable laser leaving other isotopes untouched. This is of particular importance when enriching rare and/or intermediate isotopes.

(3) Low energy consumption. LIS methods consume $\sim 10^2$–10^3 eV per separated atom (at 1–10% laser efficiency). Energy consumption per separated atom in most traditional methods is 10–10^4 times higher.

(4) Short starting time. Steady-state operating conditions for most laser methods are achieved immediately. The starting period for traditional methods can be at least several months.

(5) Possibility of running remote separation process using only laser radiation. The absence of traditional separating elements in laser methods leads to minimal contact of the enrichment material with the equipment surfaces, and hence to minimal contamination. This is particularly important for the separation of radioactive isotopes and for the management of radioactive waste of nuclear reactors.

(6) Universality. The efficiency of most traditional methods depends on the mass of the product enriched. Most laser methods can be used with almost equal efficiency for isotope separation of any element, whether light or heavy.

Experiments on a practical scale

However, not all laboratory-scale LIS methods are promising for industrial isotope separation. For industrial scaling the method should have at least two features: first, generation of laser radiation at a power level ranging from 1 kW to 1 MW (depending on the required productivity of the separation set-up). Second, laser equipment which is economical and sufficiently reliable during use.

These two requirements limit the number of LIS experiments that can be carried out on an industrial scale using existing lasers. From this point of view the most promising method is based on isotopically selective dissociation of polyatomic molecules in an intense IR field. The simplest and inexpensive gas flow TEA CO_2 lasers, with total efficiency of 5–10% and average power of up to 1 kW may be used. Initial experiments[94] on isotope separation with a pulsed CO_2 laser of average power (up to 1 kW) and high repetition rate (up to 180 Hz) have shown that comparatively high isotopic selectivity of dissociation can be obtained in these conditions.

Furthermore recent experiments[74,95] with CF_3I show that the following theoretical parameters of multi-photon isotopically selective dissociation can be realised: (1) minimum energy consumption equal to the dissociation energy of the weakest molecular bond (2 eV); (2) ultimate (almost 100%) dissociation yield of molecules in the irradiated volume at moderate energy flux of IR radiation; (3) high selectivity of dissociation >30) at comparatively high (5–10 torr) total pressure of gas; (4) significant extraction (about 50%) of the chosen rare isotope (^{13}C) without substantial decomposition (<2%) of the remaining molecules ($^{12}CF_3I$); (5) elimination of secondary photochemical processes which could destroy the high selectivity of primary dissociation. The key characteristics of multi-photon dissociation of CF_3I molecules, which are most important for carbon isotopic separation on a practical scale, are shown in Fig. 5, which shows the dependence of the primary dissociation yield of CF_3I molecules on the energy of the CO_2-laser pulse, as well as dependence of the average energy $\bar{\varepsilon}$ absorbed by the CF_3I molecule on the radiation energy. Clearly when the absorbed energy $\bar{\varepsilon}$ is greater than the dissociation energy D, almost all the CF_3I molecules are dissociated.

These results show the potential of the development of an installation for isotope separation based on a CO_2 laser. The simplicity and technological advantages of the LIS method will ensure its early industrial application.

Isotopes for nuclear energy

The isotopic enrichment of ^{235}U for power generation in nuclear reactors is the most economically important objective of LIS. World fuel requirements for nuclear reactors between now and the year 2000 are estimated to be $\sim$6 M tons of U_3O_8. Enrichment cost estimates for processing this ore amount to $\sim$150 billion US $ (refs 3, 96). An efficient laser enrichment scheme could cut these costs dramatically. Several laboratories are therefore experimenting with various schemes for LIS of uranium. As mentioned above, both the atomic process of uranium LIS[38–42] based on photoionisation and the molecular process based on photodissociation of UF_6 molecules[80–83] are being investigated very extensively.

Large quantities of D_2O are needed for heavy water nuclear reactors, which can use natural uranium fuel. The current world

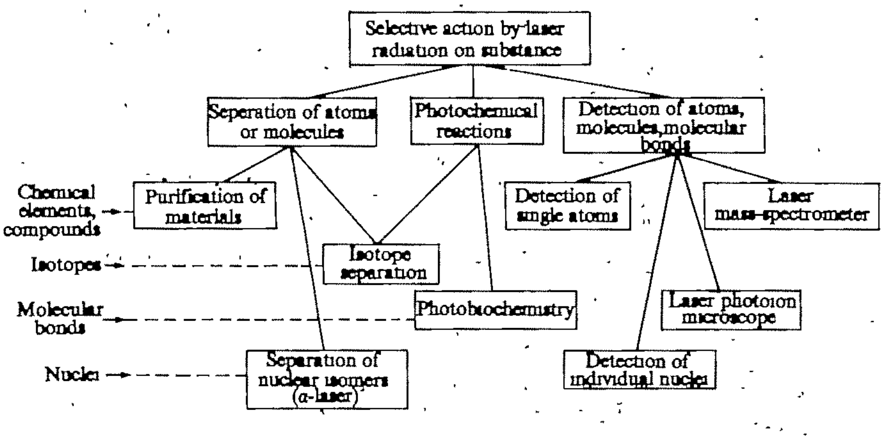
Selective action by laser radiation on substance
Separation of atoms or molecules
Photochemical reactions
Detection of atoms, molecules, molecular bonds
Chemical elements, compounds
Purification of materials
Isotopes
Isotope separation
Molecular bonds
Photobiochemistry
Nuclei
Separation of nuclear isomers (α-laser)
Detection of single atoms
Laser mass-spectrometer
Laser photoion microscope
Detection of individual nuclei

Fig. 6 Types of selective photophysical and photochemical laser processes and their applications.

production capacity is $>10^3$ ton yr^{-1}. The 800 tons of D_2O required for a 1 GW power reactor cost ~\$100 m and account for 15–20% of the total capital cost. Canadian engineers estimate that they will need more than 4×10^3 ton yr^{-1} for their reactors[97]. This means that new effective methods of hydrogen isotope separation must be found. However, it should be kept in mind that as the present production costs per atom of D are 1,000 times less than those for ^{235}U, it will be much more difficult to develop an economically viable process for D. The very low content of deuterium (1/5,000) in natural water demands rather high separation selectivity (~1,000) for economical LIS, that is several hundred times more than for uranium. Nevertheless, the possibility of hydrogen LIS by photopredissociation of HDCO using tunable UV laser radiation[97–99], and by multi-photon dissociation of polyatomic molecules by CO_2 IR laser radiation[100] is under investigation.

In the future we must also consider applying LIS methods to produce monoisotopic materials of isotopes with low-neutron capture cross-section for instance titanium isotope separation[101].

Conclusion

It would be difficult to compare in detail the different methods of laser isotope separation without considering specific isotopes or classes of isotopes. Comparison of the methods under development with those already existing is especially complicated. Laser methods are clearly better for separating isotopes that cannot be satisfactorily separated by existing methods, that is less abundant isotopes and the transuranium elements. Major technological progress in the separation of many stable isotopes in comparatively moderate quantities seems to be imminent. In the case of isotopes needed in large quantities for nuclear energy (uranium, deuterium), the situation is still somewhat uncertain. When comparing separation methods for specific isotopes we must keep in mind the level of existing laser technology. In all cases the development of large scale LIS is limited by the energy of present lasers. We need average laser powers within 10^3–10^4 W to produce only 1 ton yr^{-1}. In fact, progress in laser technology may change the situation rather drastically. An accurate prediction of the most feasible method of future large-scale laser separation of isotopes cannot be made with certainty given the present state of laser technology.

It is appropriate to emphasise that the selective photophysical and photochemical methods for laser isotope separation will find fundamental and practical application well outside the isotope separation problem. With laser radiation it is possible to use various photoprocesses (separation, chemical reactions, detection of atoms, molecules and molecular bonds) selectively in substances at atomic–molecular level with respect to various chemical elements, isotopes, nuclear isomers, molecular stereoisomers, *ortho-* and *para*-molecules, and so on[102–104]. We are essentially dealing with a new approach to the technology of materials at the atomic and molecular level when one can use laser radiation to manipulate atoms and molecules of a particular type directly—one can collect macroscopic amounts of matter 'atom by atom' and 'molecule by molecule'.

Besides selective chemical reactions and selective separation of substances at the atomic–molecular level, the process of selective detection of atoms, molecules and molecular bonds is very important. The various processes of selective laser photophysics and photochemistry are summarised in Fig. 6. Isotopically selective photophysical and photochemical processes are currently progressing very rapidly; these and their less evident applications are reviewed in ref. 104. Selective laser photoprocesses open new possibilities to related subjects, such as nuclear physics, spectroscopy, molecular biology, chemical technology and biochemistry. Some of these processes, such as laser identification of nucleotide sequences in DNA, and laser selective biochemical processes are becoming as important as isotope separation by lasers.

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articles

VLBI observations of hot spots in the lobes of distant radio sources

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In a VLBI experiment, using the Westerbork synthesis radio telescope as a total power array and the Effelsberg 100-m dish, 'hot spots' with sizes ≲0.15" have been detected in the lobes of several 3CR radio sources of high redshift. The individual lobes of double sources and the hot spots within them may have been smaller at earlier epochs.

MOST powerful extragalactic radio sources with normal radio spectra consist of two extended lobes of emission more or less symmetrically disposed with respect to the parent galaxy or quasar. Often there is also a weak compact component coincident with the optical object. In several cases high resolution observations have indicated the presence of one or more regions of enhanced surface brightness, usually near the outer edges of the radio lobes. It is generally agreed that such 'hot spots' represent regions where radio-emitting electrons are accelerated and supplied to the extended regions of the radio lobes, and several different theories have been put forward to

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explain their origin (for example, regions where beams of relativistic particles or low-frequency electromagnetic waves impinge on the intergalactic medium[1]; regions of plasma turbulence[2]; or supermassive objects ejected from the nucleus[3,4]). Detailed studies of hot spots are clearly important for an understanding of the evolution of radio sources.

Previous investigations of the statistical properties of hot spots in 3CR sources have been carried out using the techniques of aperture synthesis and of interplanetary scintillations (IPS). From synthesis observations at 5 GHz it has been reported[5] that the fractional source intensity contributed by hot spots correlates well with total source luminosity at 178 MHz, such that hot spots account for nearly all the emission in the most luminous sources. Observations of IPS at 81.5 MHz indicate[6] that the physical sizes of hot spots may increase with increasing source luminosity. But because of the strong correlation between luminosity and redshift in the 3CR sample, the properties of hot spots noted above correlate as well with redshift as with luminosity, and if the physical sizes of hot spots do not change with redshift the IPS results can also be interpreted to imply[7] a value of q_0, the deceleration parameter, in the range $0.5<q_0<2$. However, the properties ascribed to hot spots in sources at low and high redshifts may not always refer to similar physical features in their brightness distributions[8]. This is particularly true if the individual lobes of radio sources were physically smaller at earlier epochs. As the lobes of many radio sources at high redshifts ($z\gtrsim 0.5$) are often only 1 or 2 arcs in angular extent the existing aperture synthesis and IPS observations cannot indicate whether the observed components are true hot spots without any extended lobes around them or whether they have finer structure in them attributable to hot spots. Angular resolution considerably better than 1 arc s is therefore necessary to answer this important question.

We report here observations of the lobes of high luminosity 3CR sources carried out with a very-long-baseline interferometer (VLBI) system using the Westerbork synthesis array and the Effelsberg 100-m antenna. Compact components containing significant fractions of the total flux densities in sizes ≤ 0.15 arc s have been detected in the lobes of several radio sources of large redshift. These compact components have luminosities comparable to those of hot spots in nearby sources of similar total luminosity. There is, however, some indication that the physical sizes of these hot spots may have been smaller at earlier epochs, contrary to the inference drawn from the IPS results.

The source sample

A total of 35 radio sources was observed. This included 27 quasars: all those (except 3C191 and 3C207) in the 3CR sample of 200[9] that have (1) redshift $z>0.5$; (2) radio spectral index $\alpha<-0.5$ (defined as $S\propto\nu^{\alpha}$) throughout the frequency range of 80–5,000 MHz; and (3) no known low-frequency cut-off down to 38 MHz. The other eight sources observed were galaxies satisfying the above criteria, with the redshift estimated in most cases from the optical magnitudes. The sources have a luminosity $\geq 9\times10^{26}$ $\mathrm{WHz^{-1}\,sr^{-1}}$ at 178 MHz and nearly all of them are known to be standard double sources with component separations >1 arc s.

The interferometer system

The Effelsberg 100-m telescope and the 1.6-km Westerbork synthesis radio telescope used in total power mode formed the elements of the VLB interferometer for these observations at 1,417 MHz (see Table 1). The observations took place between 15.00 UT on 31 March and 15.00 UT on 2 April in 1978. The polarisation was linear in PA 0° on the sky.

Total power operation at Westerbork was achieved by coherently adding the signals from the Y dipoles of the 14 telescopes in the array after IF conversion to baseband. The added baseband signal was fed directly to the format unit of the VLBI recording terminal. A full description of the adding system is given elsewhere[10]. Steering of the telescope in delay and fringe rate (after phase offsets for the individual telescopes had been determined) was carried out in the last IF stage—the normal mode of operation for the Westerbork telescope. The fringe stopping centre, the delay centre, and the centre of the fan beam were identical on the sky. The orientation and half-power width of the fan beam are functions of the hour angle and declination of the source; at zenith on the meridian the beam width is 22 arc s east–west and 22 arc min north–south.

Table 1 Elements of the interferometer

	Antenna diameter (m)	System temperature at zenith (K)	Sensitivity (K Jy^{-1})
Effelsberg	100	75	1.5
Westerbork	93*	85	1.4

*Equivalent diameter; the Westerbork array has 14 dishes each of diameter 25 m.

Up to four short observations, each of ~10 m duration were made for each source at different hour angles within the ±6 h range at Westerbork. Observations of calibrators were interspersed at regular intervals throughout the period. In cases where the width and orientation of the Westerbork fan beam encompassed both lobes of the source, a single observation of the whole source was made. In other cases observations of each lobe were made independently.

The Effelsberg–Westerbork interferometer is 1.26 Mλ in length at the observing frequency, giving a fringe spacing of ~0.16 arc s. The length of the baseline changes very little with hour angle, but the orientation changes considerably, allowing one to say whether any particular source is non-circular in structure.

The recording of the signals was carried out with the standard Mk IIC VLBI system with a bandwidth of 2 MHz and the subsequent cross correlations and data reduction were done at the Max Planck Institute for Radio Astronomy. A hydrogen maser oscillator at Effelsberg and a rubidium oscillator at Westerbork provided the time and frequency standards.

Calibration of the data

The correlated flux density scale was calibrated using the sources NRAO140, 4C39.25, OQ208, 3C345 and 3C454.3. Table 3 lists the calibrator sources, the number of times they were observed, the total flux density as measured at Effelsberg during the observations and the flux density in compact components. In NRAO140 and OQ208 the compact components supply the total flux density. Measurements of the larger scale structure in 3C345 and 3C454.3 by Davies *et al.*[11] were used to remove the contribution of these steep-spectrum resolved components to the total flux density. Available spectral information on 4C39.25 was used to remove the contribution of a steep-spectrum component. The calibration factor for all five sources has an r.m.s. scatter of ~6%. Removing OQ208 reduces the r.m.s. scatter to ~2%; this implies that the measured flux for OQ208 may be too low. The measured r.m.s. scatter in amplitude from one scan to another for the individual calibrators is ~4%.

Checks on the calibrator sources indicated that there was no loss in fringe amplitude due to phase drifts in the oscillators (primarily the rubidium) on time scales of 10 m. Consequently,

Table 2 Geocentric coordinates of the observatories

Observatory	X (km)	Y (km)	Z (km)
Effelsberg	4,033.778	−486.995	4,900.529
Westerbork	3,828.674	−443 226	5,064.916

The X-axis points to latitude 0°, longitude 0°; the Y axis to latitude 0°, longitude 90°; and the Z axis to latitude 90°

each observation on the extended sources was integrated coherently for the duration of the observation, typically 10 m. The r.m.s. noise in correlated flux density for a 10 m coherent integration was observed to be 3 to 4 mJy, allowing reliable detections of compact components as weak as 15 to 20 mJy.

The X and Y components of the baseline were determined by least-squares fitting to the fringe rates for the calibrators, and a crude estimate of the Z component was made from the delay function corresponding to the 2 MHz recorded bandwidth. The X, Y, Z parameters of the individual telescopes are given in Table 2. The r.m.s. scatter in the fringe rates of the calibrators about zero residual fringe rate is ±1 mHz assuming the best fit baseline. This means that the pointing of the beam centre is known to that accuracy in the fringe rate variable. This is sufficient to distinguish, in many of the sources observed, between a compact component at the centre of the beam and one in an outer lobe.

The width and orientation of the Westerbork beam were calculated for each observation. In cases where components were detected away from the beam centre their flux densities were scaled according to their positions in the beam.

Correlated flux density from hot spots and nuclear components

The correlated flux density for each observation was determined from the fringe frequency spectrum obtained by transforming all the data for that observation. A comparison of the residual fringe frequency for each significant peak in the spectrum with the expected values for components in the individual lobes and at the location of the optical object enabled unambiguous identification of the observed VLBI components in most sources in which the lobes are separated by $\gtrsim 10$ arc s.

Correlated flux density exceeding a signal-to-noise ratio of 5 was detected in all the sources observed except 3C41 for which only a single 10 m observation was available. The results for sources in which at least one hot spot is likely to have been detected in the outer lobes are presented in Table 4a. The observed components have been identified in Table 4 by the letters A, B or N which refer respectively to the western lobe, the eastern lobe and the nuclear component associated with the optical object. Other relevant information listed in Table 4 is the optical identification, the redshift, the angular separation between the outer lobes, θ_{AB} (taken from ref. 12 and references therein), the measured total flux density at 21 cm and the flux density in the nuclear component at 6 cm as measured by the Cambridge 5 km telescope. A blank in column 6 implies that a nuclear component was not detected at 6 cm and a question mark implies that flux density in the nuclear component cannot be determined unambiguously. The number of different hour angles at which the VLBI observations were made is given in column 7 and the maximum correlated flux density for each component is listed in column 8. A more detailed table giving the measurements at each observed U, V point will be published elsewhere. The errors quoted in column 8 are a quadratic combination of 4% of the total flux density and the r.m.s. noise for that observation.

Table 3 The calibrators

Source	No. of scans	Measured total flux density (21 cm)* (Jy)	Unresolved flux density (Jy)
NRAO 140	2	3.66	3.66
4C39.25	2	2.53	2.05
OQ208	5	0.81	0.81
3C345	2	7.39	6.77
3C454.3	1	10.86	10.23

*For an assumed sensitivity of 1.51 ± 0.06 K Jy^{-1}. This value was derived from the measured antenna temperatures at Effelsberg for all the 3CR sources in the sample and using the total flux densities in Bridle et al.[26].

Sources for which the observed VLBI components are likely to be associated with nuclear components are listed in Table 4b. As can be seen from Table 4a and b the correlated flux density measured by us in the nuclear components at 21 cm is in general less than or only slightly more than that measured at 5 GHz with the Cambridge 5-km telescope. This agrees with the generally accepted result that most nuclear components have flat or inverted radio spectra at these frequencies. We also find that the variation in correlated flux density with hour angle for the nuclear components is in general small, implying that these components are unresolved with the present baseline.

In a few sources, mainly those for which the overall radio structure is not well determined, it is not possible to make unambiguous identifications of the observed VLBI components with known features in their brightness distributions. In the case of 3C43 and 3C454 the correlated flux density shows little variation with hour angle and corresponds to ~26% and ~16% of the total flux at 21 cm, respectively. The overall sizes of the sources are <1 arc s and the total spectra are straight between 38 MHz and 5 GHz with no sign of a flattening at high frequencies[13]. The observed VLBI components in these sources are therefore unlikely to be associated with flat spectrum nuclear components.

In the case of 3C186 only the northern component (A) of the 100 arc s double[14] was observed. This component lies close to the QSO and could itself be double with a component separation of 1.8 arc s (ref. 15). It is uncertain whether the weaker southern lobe is physically related to the QSO. The maximum observed correlated flux density is ~22% of the total at 21 cm and is unlikely to arise in a flat spectrum nuclear component as there is no observed flattening of the total spectrum at high frequencies[13].

The overall size of 3C216 is <1 arc s and the total spectrum which seems to flatten out at frequencies above 1.4 GHz (ref. 13) is consistent with all the correlated flux arising in a flat spectrum nuclear component.

3C245 ($\theta_{AB} = 5''$) has a D2 type structure[16]; the component B being coincident with the QSO[12]. The correlated flux density corresponds to ~47% of the total and could be associated with component B.

A map of 3C380 at 15 GHz (ref. 17) shows this source to be a double with a component separation of ~1.2 arc s, the stronger component possibly coinciding with the QSO. The VLBI component seems to be partly resolved and could be associated with the nuclear component.

Comparison with Cygnus A

It is interesting to compare the properties of hot spots in sources of large redshift with those in the well observed source Cygnus A, which is a relatively close radio galaxy ($z = 0.056$) with an intrinsic luminosity similar to that of the QSOs observed here. Aperture synthesis observations at 5 GHz indicate[18] the presence of four hot spots in Cygnus A with angular sizes between ~1 and 2 arc s. From the available spectral information it is estimated that at 1.4 GHz the hot spots together should account for about 19% of the source flux density, most of which is contributed by two most prominent hot spots numbered A (~7%) in the Np lobe and D(~10%) in the Sf lobe[18]. These spots have physical sizes between about 2 and 3 kpc. Higher resolution observations at 15 GHz (ref. 19) indicate little significant structure on a more compact scale.

From the present VLBI observations it is not possible to estimate the exact shapes of the hot spots or the fractional source flux density which they contain. As the length of the projected baseline does not change significantly over the hour angle range of these observations, the interferometer is sensitive to similar angular scales in most sources. At the mean projected baseline of $1.2 \times 10^6\,\lambda$ the normalised fringe visibilities of gaussian structures with half-intensity diameters of 0.05, 0.1 and 0.15 arc s would be about 0.74, 0.30 and 0.07, respectively. The

Table 4 *a*, Sources with hot spots; *b*, sources in which only nuclear components were detected

Source	Optical identification	Redshift	θ_{AB} (arc s)	S_{tot}(21 cm) (Jy)	$S_{nuclear}$(6 cm) (mJy)	No. of scans	Maximum S_{corr}(21 cm) (mJy)
a							
3C6.1	G	(0.75)	26	3.32	20±10	1	72±5 (component B)
9	Q	2.012	10	1 93	—	3	28±4 (B or N)
13	G	(0.5)	28	1.80	—	3	140±7 (A) 145±7 (B)
43	Q	1.459	<1.5	2.79	?	3	773±31*
55	G	(0.75)	72	2.38	—	3	62±5 (A)
68.1	Q	1.238	46	2.45	—	4	34±5 (A)
175	Q	0.768	48	2.45	40±8	2	20±4 (B)
181	Q	1.382	6	2 29	—	3	179±8 (A) 332±14 (B)
186	Q	1.063	100	1.35	?	4	291±12 (A)*
196	Q	0.871	5	13.84	—	4	532±22 (A more likely than B)
205	Q	1.534	16	2.29	30±10	3	317±13 (A)
254	Q	0.734	13	2.83	—	3	112±6 (A) 64±5 (B)
263	Q	0.652	44	3.07	130±20	2	264±11 (B) 173±8 (N)
268.4	Q	1.40	10	2 10	50±20	4	503±21 (A)
270.1	Q	1 519	12	2.59	?	4	290±12 (B+N)
275.1	Q	0.557	14	2.89	—	3	70±5 (A) 116±6 (B)†
280	G	(0.5)	13	5.00	—	4	356±15 (A)
280.1	Q	1.659	22	1.38	55±10	3	23±4 (A) 45±5 (N)
288.1	Q	0.961	7	1.51	—	3	39±4 (A) 44±4 (B)
330	G	0.549	56	6.62	—	1	46±4 (A more likely than B or N)
352	G	(0.5)	10	1.84	—	3	49±5 (A, B or N)
432	Q	1.805	13	1 56	—	3	45±4 (B more likely than A)
454	Q	1.757	<1.3	1.96	?	3	333±14*
455	Q	0.543	3.2	2.76	?	3	75±5 (A or B)
b							
3C200	G	(0.5)	17	2.02	88±15	4	19±4
204	Q	1.112	31	1.30	40±10	4	82±5‡
208	Q	1.109	11	2.42	54±5	1	79±6
212	Q	1.043	9	2.50	310±50	1	85±6
216	Q	0 67	0.8	3.88	?	2	554±23*
220.2	Q	1.157	8	1.75	60±15	3	118±6†
245	Q	1.029	4.6	3.18	1,200±100	2	1,485±60*
334	Q	0.555	48	1.96	170±20	2	180±8
336	Q	0.927	22	2.48	30±10	3	19±5
380	Q	0.691	1.2	13.63	?	3	4,033±160*

*See text. †May include some contribution from a nuclear component. ‡May include some contributions from A and B.

maximum correlated fluxes listed in Table 4 are therefore in most cases likely to be lower limits to the flux densities contained in hot spots while the angular diameters of the hot spots are likely to be ≤ 0.15 arc s, which corresponds to ≤ 1.3 kpc at $z = 1$ (for $H = 50\ \mathrm{km\ s^{-1}\ Mpc^{-1}}$ and the Einstein–de Sitter world model), typical of the sources observed.

It is clear from a histogram of the normalised maximum visibility (γ_{max}) for all the sources observed here (Fig. 1*a*) that in over one-third of them the hot spots are responsible for at least 5% of the source flux density, and in this respect are not very different from those in Cygnus A. The physical sizes of these hot spots are, however, likely to be smaller than those of Cygnus A. Hot spots of size >2 kpc at $z > 0.5$ would, in fact, have been completely resolved out in the present experiment. It is therefore possible that hot spots similar to those in Cygnus A are even more common in high redshift sources than is apparent from the present number of detections.

In view of the limited U, V coverage of the observations it is important to ask if the observed visibilities could arise from highly non-gaussian brightness distributions such as uniform disks or linear structures with sizes >0.15 arc s. A circular disk in our case would have a secondary maximum in the visibility curve for a diameter of 0.3 arc s with $\gamma \sim 0.13$. As seven sources have $\gamma_{max} > 0.13$, such distributions are unlikely to be true in general. Higher visibilities at the secondary maximum are possible for linear brightness distributions but the values of γ would be strongly dependent on the hour angle of observation due to projection effects. Only in three of the 13 sources with $\gamma_{max} > 0.05$ was the ratio of γ_{max} to γ_{min} found to be >2 and only in one case >3. As the sources were observed typically at three different position angles differing by over 90° on the sky, such highly elongated components seem to be unlikely unless their maximum dimensions are appreciably smaller than the fringe separation of the interferometer.

The minimum equipartition energy requirements in the hot spots of Cygnus A have been estimated[18] to be of the order of 3×10^{57} erg. It was also shown by Hargrave and Ryle[18] that for inferred equipartition magnetic field of $\sim 3 \times 10^{-4}$ G the radiation lifetime of 5 GHz electrons is only $\sim 5 \times 10^{4}$ yr and that continuing acceleration of electrons must therefore take place in the hots spots and the particles must diffuse out of them rather quickly. These arguments are also valid for the hot spots seen in more distant sources. As the total luminosities of many of the hot spots reported here are comparable with those in Cygnus A, the magnetic field should scale as $V^{-2/7}$, whereas the minimum total energy and the electron lifetimes should scale as $V^{3/7}$, where V is the volume of the hot spots. Because the volumes of the hot spots reported here could be more than 10 times smaller than Cygnus A the electron lifetimes at radio frequencies would be even smaller than in the case of Cygnus A.

Cosmological implications

There is considerable evidence from the observed angular size–redshift relationship for QSOs (see ref. 20) and the angular size–flux density relationship[21,22] that the lobe separations of double radio sources were smaller at earlier epochs. It is important to know if such evolutionary effects may also be present in the sizes of individual lobes of radio sources and of hot

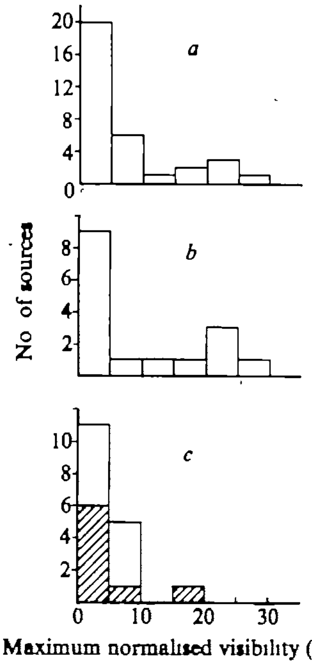

Fig. 1 The distribution of maximum observed correlated flux densities from hot spots, normalised with respect to the total source flux densities, in: *a*, all the sources; *b*, sources with $z > 1$ and *c*, sources with $z < 1$. Sources in which hot spots were not detected have been included in the first bins (0 to 5%). 3C216 and 3C380 have not been included in the histograms and galaxies are shown hatched.

spots within them. A plot of individual lobe sizes with redshift for the 3CR sources shows[8] that at high redshifts ($z > 0.5$) most sources have lobe sizes of only 1–2 arc s which is considerably smaller than expected in reasonable world models if the lobes had similar physical sizes to those at lower redshifts. Our detection of compact hot spots similar in intensity to those in Cygnus A in many high redshift sources implies that the component sizes measured by the Cambridge 5 km telescope for these sources refer to the lobes and not to the hot spots within them. It is therefore likely that the physical sizes of individual lobes of double radio sources undergo similar evolutionary effects with epoch as the lobe separations.

The detection of hot spots of size $\lesssim 0.15$ arc s in high-luminosity sources also suggests that the strong correlation between the fractional flux in hot spots and total source luminosity suggested by Jenkins and McEllin[5] for the 3CR sources with Class II[23] radio structure, suffers from insufficient resolution of the synthesis observations. For this correlation Jenkins and McEllin defined hot spots as regions with a size <15 kpc, which corresponds to an angular size <2 arc s for redshifts $\gtrsim 0.5$. As most radio sources at $z < 0.3$ have measured lobe sizes > 15 kpc and those at $z > 0.5$ have lobes <15 kpc (see ref. 8) the 2 arc s resolution of the synthesis observations is insufficient to distinguish the contribution of hot spots from that of the more extended lobes at higher redshifts (or higher luminosities). But due to the criterion used to define hot spots nearly all the source flux density is considered to arise in hot spots. This could be responsible for much of the observed correlation. Because of the very different angular resolutions it is difficult to compare the fractional flux in hotspots derived from synthesis observations of nearby sources with that detected by VLBI in the present high redshift sample. However, it is interesting that the fractional flux in hotspots at 5 GHz for the 10 sources in the table of Jenkins and McEllin[5] with $z < 0.1$ varies between 5 and 38%, with an average value of 14%, not significantly different from the VLBI visibilities seen in the high redshift sources.

Table 5 Comparison of VLBI and IPS results

	VLBI		IPS	
Source	$\gamma_{\max}$	$\gamma_{\min}$	R	ψ (arc s)
3C43	0.28	0.24	0.51	0.26
3C181	0.22	0.10	0.39	0.47
3C186	0.22	0.09	0.51	0.32
3C196	0.04	0.01	0.33	0.70
3C254	0.06	0.05	0.33	0.64
3C268.4	0.24	0.14	0.51	0.50
3C270.1	0.11	0.05	0.24	0.14
3C280.1	0.02	<0.02	0.45	0.70

If the physical sizes of hot spots in Cygnus A are typical of low redshift sources, as is indicated by IPS observations[6], then the present detection of hot spots with sizes <1 kpc in several of the high redshift sources suggests that the hot spot sizes may also have been smaller at earlier epochs. Some further support for such a possibility may be seen within the present source sample if the sources are divided into two groups depending on whether these have redshifts greater than or less than 1, as shown in Fig. 1*b*, *c*. Although the sample is small the values of $\gamma_{\max}$ seem to be higher in the $z > 1$ sample, as might be expected if the hot spots were more compact at higher redshifts. The observations are, however, also consistent with a weak correlation between the fractional flux in hot spots and redshift.

The possible evolutionary effect indicated by the present observations is contrary to the conclusions of Hewish *et al.*[7] based on IPS observations. Their plot of the inferred angular diameter of hot spots for a sample of 23 strongly scintillating 3CR sources against redshift indicated a strong deficit of sources with diameters <0.3 arc s at redshifts >0.7. This was interpreted by them to imply either that $0.5 < q_0 < 2.0$, or if q_0 is to be smaller, the linear size of hot spots must increase with redshift. But the present VLBI measurements make it doubtful if the angular diameters derived from IPS for high redshift sources refer to true hot spots. This may be seen from a comparison of the VLBI and IPS observations for the eight sources that seem to be common to the two samples at $z > 0.7$ (the IPS data are from ref. 6). Table 6 shows the parameters R and ψ (which are measures of the fractional flux density and the angular diameter of the scintillating component respectively) derived from IPS, together with the values of $\gamma_{\max}$ and $\gamma_{\min}$ in hot spots observed by VLBI.

Based on a new determination of the spectrum of electron density fluctuations in the solar wind, Readhead *et al.*[27] have recently pointed out that the published values of R and ψ should be reduced by ~40% and ~25%, respectively. The revised values have been used in Table 5. The values of ψ quoted in Table 5 have been further reduced by ~20% to convert the e^{-1} full widths used to define the size in ref. 6 to full widths at halfpower. In six of the eight sources, hot spots with angular diameters $\lesssim 0.15$ arc s contain at least 6–28% of the source flux density. The diameters derived from IPS for these sources, on the other hand, are generally >0.3 arc s. The angular diameters of the individual lobes as determined directly from synthesis observations with the Cambridge 5-km telescope are in most cases less than or of the order of 2 arc s. As all structures smaller than about 2 arc s contribute to IPS at 81.5 MHz, the diameters inferred from IPS are likely to be suitably weighted means of the hot spot and lobe sizes. The importance of such a blending of source components in interpreting the IPS results was pointed out earlier by Swarup and Bhandari[24], but it was noted by Hewish and Readhead[25] that the estimated values of R for sources of large z are considerably higher than would be expected if blending were important. The downwards revision by 40% in the values of R, however, makes them consistent with the blending hypothesis.

The present VLBI observations together with the overall source structures determined by aperture synthesis thus suggest that the individual lobes of double sources and perhaps also the hot spots within them were more compact at earlier epochs. Because of the tight correlation between redshift and luminosity in the 3CR sample it is not, however, possible to rule out the dependence of component sizes on absolute luminosity rather than on cosmological epoch. Observations of much weaker samples to see lower luminosity sources at high redshifts are needed to decide between the two possibilities. High-resolution mapping of compact structures in the radio lobes may also throw light on the physical nature of the evolutionary mechanism.

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Isotopes of tellurium, xenon and krypton in Allende meteorite retain record of nucleosynthesis

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Various mixtures of r-, p- and s-nucleosynthesis products have been observed in the isotopes of tellurium, xenon and krypton extracted from mineral separates of the Allende meteorite. The presence of several isotopically distinct components in these high Z elements and the close association of isotopically normal low Z elements with particular isotopes of the high Z elements suggest that our Solar System may have formed directly from the debris of a single precursor star, approximately concentric with the present Sun.

THE enrichment of heavy xenon isotopes in carbonaceous chondrites is always accompanied by an enrichment of the light, fission-shielded isotopes. It has been suggested that this anomalous xenon might represent a mixture of r- and p-products added to the Solar System from a nearby supernova[1]—previously the excess heavy isotopes were widely regarded as fission products, designated CCF as an acronym for carbonaceous chondrite fission[2]. More recent reports of nucleogenetic anomalies in the isotopic compositions of several other elements in meteorites[3–10] and identification[11] of the decay product of extinct ^{26}Al have resulted in three different models of element synthesis shortly before condensation: (1) The anomalous elements and short-lived isotopes might have been generated in remote nucleosynthesis events, such as supernovae[12,13] or red giant stars[14,15], and then trapped in grains which were subsequently incorporated into the early solar nebula. In such a case the short-lived isotopes themselves would not necessarily survive until the grains entered the solar nebula, but the more refractory dust grains might survive conditions in the nebula and be incorporated into meteorites with alien nucleosynthesis products and the decay products of short-lived isotopes. (2) The anomalous elements and short-lived isotopes might have been generated in a nearby supernova[16]. If the shock wave from this supernova caused collapse of the presolar nebula, then the nucleosynthesis event is directly related to the birth of the Solar System and occurred about 4.6×10^9 yr ago. (3) The Solar System might have formed directly from the debris of a single supernova[17–19]. In this case the anomalous elements and short-lived isotopes were made by the same nucleosynthesis events which produced other elements in the Solar System, and the present Solar System retains the chemical characteristics of the supernova: The Sun accumulated on the supernova core, iron meteorites and cores of the terrestrial planets were formed primarily from elements synthesised in the hot stellar interior, and the outer planets and the carbon phase of chondrites were condensed from elements of the cooler outer layers, the only region which contained low-Z elements.

Two recent reviews[20,21] suggest that model (2), a nearby supernova, has gained favour as a reasonable working hypothesis. However, the results of analyses on noble gases trapped in the metal phase of iron meteorites[22] and in gas-rich xenoliths[23] from the Earth's mantle have been interpreted as supporting model (3). Other tests were suggested for model (3), including its prediction that the acid-resistant carbon which contains anomalous xenon should also contain isotopically anomalous components of other high-Z elements[22]. Subsequently, Arden[6] has reported large isotopic anomalies of uranium in acid-resistant minerals of several chondrites, but it was uncertain whether these were generated by stellar nuclear reactions or by chemical fractionation of the precursor elements of ^{235}U and ^{238}U. The present study was therefore undertaken to test the prediction of model (3) at tellurium ($Z=52$), an element with an atomic number near that of xenon ($Z=54$) and with eight natural isotopes that are only inefficiently produced by fission.

Srinivasan followed procedures developed elsewhere[24–27] to isolate the following acid-resistant fractions from a crushed sample of bulk Allende weighing 11.66 g. (*a*) Residue A: Etch-

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Table 1 Ratios of stable xenon isotopes

	Carbon (C-2)				Spinels and carbon (B-1)			Spinels*		
Isotope	800°	1,200°	1,600°	Total	800°	1,600°	Total	Total	AVCC	Air
^{124}Xe	0.497 ±0.005	0.564 ±0.008	0.572 ±0.007	0.548 ±0.004	0.476 ±0.006	0.485 ±0.008	0.478 ±0.006	0.355 ±0.018	0.459	0.357
^{126}Xe	0.447 ±0.008	0.458 ±0.007	0.456 ±0.006	0.454 ±0.005	0.417 ±0.005	0.409 ±0.018	0.415 ±0.008	0.348 ±0.024	0.410	0.335
^{128}Xe	8.29 ±0.08	8.42 ±0.08	8.44 ±0.11	8.39 ±0.06	8.29 ±0.06	8.02 ±0.17	8.24 ±0.08	7.97 ±0.24	8.20	7.14
^{129}Xe	140.3 ±0.8	106.1 ±0.6	105.2 ±0.6	115.2 ±0.4	116.6 ±0.5	102.3 ±0.6	113.8 ±0.5	111.4 ±1.6	~100	98.3
^{130}Xe	16.13 ±0.11	15.90 ±0.09	15.98 ±0.09	15.99 ±0.07	16.28 ±0.09	15.84 ±0.10	16.19 ±0.09	16.55 ±0.28	16.08	15.17
^{131}Xe	81.55 ±0.26	82.24 ±0.29	82.21 ±0.37	82.04 ±0.23	81.95 ±0.32	80.86 ±0.62	81.74 ±0.38	81.21 ±1.12	81.7	78.77
^{132}Xe	=100	=100	=100	=100	=100	=100	=100	=100	=100	=100
^{134}Xe	38.44 ±0.15	45.21 ±0.24	43.92 ±0.17	42.98 ±0.12	38.15 ±0.18	40.87 ±0.18	38.68 ±0.18	31.16 ±0.54	38.2	38.82
^{136}Xe	32.41 ±0.23	42.74 ±0.30	40.97 ±0.20	39.39 ±0.12	32.07 ±0.22	36.13 ±0.29	32.86 ±0.23	21.45 ±0.67	32.1	32.99
^{132}Xe content (cm^3 STP g^{-1})$\times 10^{-8}$	1.22	1.94	1.29	4.45	2.60	0.62	3.22	2.17	—	—

* (Amount of isotope in spinels) = (Amount of isotope in B-1) − (Fractional wt of C in B) $\frac{\text{wt of B-1}}{\text{wt of C-2}}$ (Amount of isotope in C-2).

ing bulk Allende in HCl and HF acids for a 6-week period produced residues weighing 0.53% of the bulk sample. Previous studies[24–27] on Allende residues suggest that carbon, chromite, spinel and an ill-defined mineral Q are the main constituents of residue A. (*b*) Residue B: Etching residue A in concentrated HNO_3 dissolved Q (refs 26, 27), leaving a residue weighing 0.42% of the bulk Allende sample. (*c*) Residue C: Refluxing residue B in a mixture of concentrated acids, H_3PO_4 and H_2SO_4, for 2 h dissolved[28] the chromite and spinel (and perhaps part of the carbon) to produce residue C, weighing 0.19% of the bulk sample. (*d*) Dissolution of C: Refluxing residue C for 1 h in a mixture of concentrated acids, H_3PO_4 and H_2SO_4, plus CrO_3, dissolved residue C.

Nucleosynthetic products in xenon and krypton

A high sensitivity noble gas mass spectrometer was used to determine the isotopic compositions of xenon and krypton released by stepwise heating of a 3.1-mg aliquot of residue B, labelled B-1, and a 1.7-mg aliquot of residue C, labelled C-2. Procedures for extraction and analysis of noble gases were as described previously[22]. The isotopic compositions of xenon and krypton in these residues are presented in Tables 1 and 2, respectively. The xenon in both residues displays the now familiar pattern of enrichment in the light and heavy isotopes.

The part (chromite, spinel and perhaps some carbon) of residue B which dissolved (54% by weight) in producing residue C is referred to collectively as spinels in Tables 1 and 2. The concentrations and isotopic structures of gases in these spinels were calculated from differences in the isotopic compositions and total gas contents of residues B and C. Superficially the isotopic composition of xenon and krypton in Allende spinels is similar to that reported recently in the high temperature fractions of Murchison residues which were etched in alkaline oxidants to remove organic polymers[15]. It was reported that X-ray diffraction of the severely etched Murchison residues showed mainly chromite and minor spinels—minerals which probably constitute the bulk of our Allende spinels. As was observed in the high temperature fractions of these Murchison residues[15], the xenon in Allende spinels is enriched in ^{130}Xe and depleted in ^{136}Xe, and the krypton in these spinels seems to be enriched in ^{82}Kr and depleted in ^{83}Kr. However, the isotopic composition of Kr in our spinels cannot be distinguished from bulk krypton in carbonaceous chondrites or in air, within the limits of experimental error. A detailed examination of the xenon spectrum in Allende's spinels is instructive in evaluating the model proposed to explain the high temperature xenon in Murchison's spinel—s products trapped in presolar dust grains that were ejected from red giant stars[14,15].

The isotopic spectra of xenon in Allende's carbon and spinels are shown in Fig. 1, normalised to xenon in average carbonaceous chondrites (AVCC) in the following manner.

$$g^{i}_{132} = ({}^{i}Xe/{}^{132}Xe)_{sample}/({}^{i}Xe/{}^{132}Xe)_{AVCC} \qquad (1)$$

Since the *in situ* decay product of ^{129}I may comprise part of the

Table 2 Ratios of stable krypton isotopes

	Carbon (C-2)				Spinels and carbon (B-1)			Spinels*		
Isotope	800°	1,200°	1,600°	Total	800°	1,600°	Total	Total	AVCC	Air
^{80}Kr	4.22 ±0.06	3.95 ±0.04	3.92 ±0.07	4.05 ±0.04	4.09 ±0.07	3.87 ±0.04	4.02 ±0.06	3.98 ±0.20	3.92	3.96
^{82}Kr	20.45 ±0.17	19.72 ±0.05	19.32 ±0.03	19.88 ±0.09	20.25 ±0.28	19.75 ±0.17	20.09 ±0.25	20.51 ±0.75	20.14	20.21
^{83}Kr	20.42 ±0.15	20.11 ±0.11	20.00 ±0.10	20.20 ±0.12	20.17 ±0.10	19.92 ±0.08	20.09 ±0.09	19.88 ±0.36	20.17	20.16
^{84}Kr	=100	=100	=100	=100	=100	=100	=100	=100	=100	=100
^{86}Kr	30.33 ±0.27	31.44 ±0.15	31.48 ±0.19	30.91 ±0.21	30.55 ±0.14	31.24 ±0.15	30.77 ±0.14	30.49 ±0.59	30.98	30.55
^{84}Kr content (cm^3 STP g^{-1})$\times 10^{-8}$	4.13	2.89	3.43	10.45	4.98	2.28	7.26	4.53	—	—

* See Table 1 footnote for method used to calculate amount of each isotope in spinels

^{129}Xe abundance, this isotope is not used to define the general anomaly pattern of isotopes trapped in Allende's spinels and carbon. Abundances of the other xenon isotopes in carbon and spinels display opposite anomaly patterns. We suggest that these mineral fractions trapped different components of AVCC xenon, that is, carbon is enriched in r and p products and spinels are enriched in s products of the nucleosynthesis reactions which collectively produced the isotopes of bulk xenon in carbonaceous chondrites. It seems highly unlikely that the carbon and spinel fractions are both interstellar grains, or that either the carbon or the spinels forming in the primitive nebula were selectively exposed to alien xenon from nearby supernovae or red giant stars. There are other indications that noble gases in Allende do not represent alien nucleosynthesis products. Allende's carbon contains helium and neon with normal planetary isotopic compositions[29], and the carbon itself has a normal value of ^{12}C/^{13}C (ref. 30). The ^{82}Kr/^{130}Xe ratio in Allende's spinels is 2.6, which is close to the value of ^{82}Kr/^{130}Xe = 1.8 in bulk Allende[26] and about a factor of 10 higher than the value that Srinivasan and Anders[15] report for this ratio in the gas component from red giant stars.

Variations in the abundance of the lightest and heaviest xenon isotopes in gas released by stepwise heating of Allende's acid-etched residues are shown graphically in Fig. 2. Xenon isotopes released from the carbon (C-2) and spinel/carbon (B-1) fractions define different correlation lines. These do not agree with the correlation shown by Lewis *et al.*[26,27] for other etched residues of Allende (dashed line in Fig. 2), nor do they pass through the composition of AVCC or 'trapped' (ref. 26) xenon. Since ^{132}Xe may be produced by both r- and s-processes[31], it is preferable to normalise the xenon spectra to a pure s-product, ^{130}Xe.

As shown in Fig. 3*A*, this method of normalisation permits us to compare trends in isotopic ratios with trends expected from the addition of pure s-, r- and p-nucleosynthesis products to AVCC xenon. It can be seen in Fig. 3*B* that xenon isotopic ratios from stepwise heating of our two Allende residues do not follow the trend reported earlier[26,27] (dashed line in Fig. 3*A*), but it can be seen in Fig. 3*C* that ratios for the total xenon in these residues seem to do so. Isotopic ratios of the total xenon in other etched residues of Allende[26,27] also lie along the dashed line shown in Fig. 3*A*, as expected by mixing only two components. But isotopic ratios of xenon released by stepwise heating of individual residues from Allende and Murchison, as shown in Fig. 3*D*, *E* and *F*, cannot be explained by a simple binary mixture. Kuroda[32] also notes that a simple binary mixture fails to explain isotopic ratios of xenon released by stepwise heating of bulk carbonaceous chondrites. Although the spectra in Fig. 3 confirm the positive correlation between enrichments of light and heavy xenon isotopes[1], and the spectra in Fig. 1 suggest that bulk AVCC xenon may be represented as a mixture of one s-enriched component with one s-depleted component, differences in the slopes of the correlation lines in Fig. 3 indicate that the r and p products of chondritic xenon were not completely mixed before trapping. Since the r and p processes occur in the supernova explosion[31], to explain these xenon spectra as a consequence of injections from a nearby supernova[1,16] would require the preservation of heterogeneities within the supernova material itself as the alien xenon was transferred into the solar nebula and then trapped on a matrix of isotopically normal carbon.

Variations in the isotopic composition of krypton (Table 2) provide additional evidence for nucleogenetic effects in the residues of Allende. Since the anomalies in krypton are relatively small and others[15,33] have recently discussed the evidence for s- and r-products in krypton extracted from acid-etched minerals of carbonaceous chondrites, a detailed discussion on the krypton anomalies shown in Table 2 is not warranted.

Nucleosynthetic products in tellurium

If Allende's carbon itself formed in the outer layers of a supernova, then other heavy elements trapped in the carbon would be expected to show enrichments of r- and p-products similar to those observed in xenon. To test this, the abundance of six stable isotopes, ^{i}Te, i = 120, 122, 124, 126, 128 and 130, was determined in Allende's residues by neutron activation and γ-ray spectrometry on the (n, γ) product ${}^{i+1}$Te. For these analyses a 32-mg aliquot of residue B, labelled B-3, was irradiated for 95 h at a flux of 6×10^{14} neutrons cm^{-2} s^{-1}. Monitors of tellurium, uranium, osmium and ruthenium were irradiated with the sample. Carriers of tellurium, iodine (to carry fissiogenic ^{133}I), osmium and ruthenium were added to the irradiated sample during etching to isolate the activation products from fractions of the residues rich in spinels and carbon, respectively. Standard radiochemical procedures were used to recover tellurium[34–38] and iodine[39], and to recover and separate osmium and ruthenium[35,40–45]. The only special reagent, potassium chlormilexathiocyanate, was prepared as described by Morgan[45] and

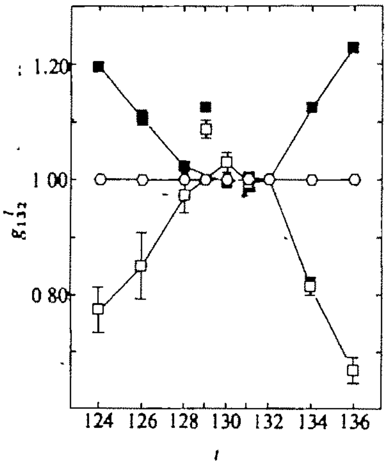

Fig. 1 Isotopic compositions of xenon in Allende's spinels (□) and carbon (■) normalised to AVCC xenon (⬡) in the manner given by equation (1). Since ^{129}Xe may contain the *in situ* decay product of ^{129}I, it is not used to define the isotopic abundance pattern of trapped xenon. Carbon is enriched in r and p products of xenon, spinels are depleted in these, and bulk AVCC xenon has an intermediate abundance pattern for the eight nonradiogenic isotopes of xenon.

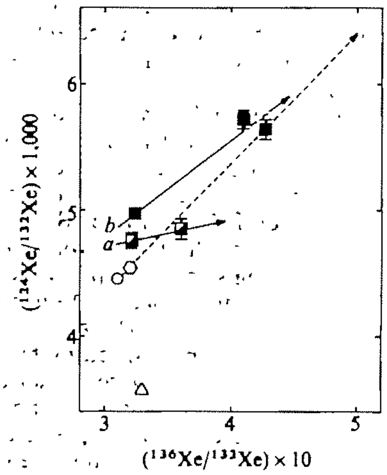

Fig. 2 Variations in abundances of the lightest and heaviest isotopes of xenon released by stepwise heating of Allende's residues. Xenon isotopes released from the mixed residue of carbon and spinels (◪) lie along line *a*, xenon isotopes from the carbon residue (■) lie along line *b*, and xenon isotopes in other residues of Allende are reported[26,27] to lie along the dashed line connecting 'fission-free' or 'trapped' xenon (○) and 'strange' xenon generated by fission and mass fractionation. ⬡, AVCC; △, air.

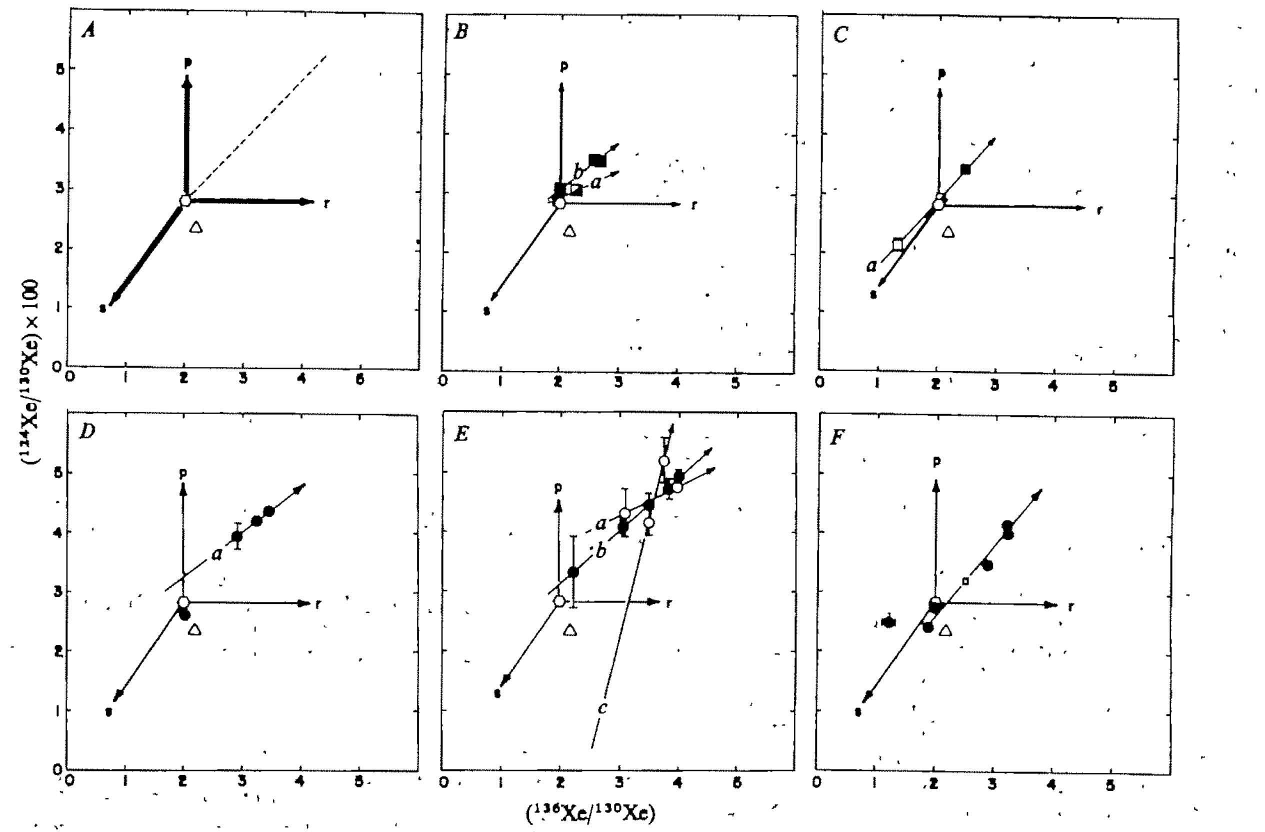

Fig. 3 Ratios among three xenon isotopes produced by the r, p and s processes of nucleosynthesis. *A*, A comparison of trends expected by the addition to AVCC xenon of pure nucleogenetic components from the r, p and s processes (dark arrows) with trends reported from earlier analyses[26,27] on Allende residues (dashed line); *B*, trends observed in this study for xenon released by stepwise heating of Allende's carbon (C-2) (line *b*) and carbon plus spinels (B-1) (line *a*); *C*, trends observed in this study for total xenon in Allende residues: *a* is B-1, C-2 mixing line; *D*, the trend observed in an earlier study[26] on xenon released by stepwise heating of one Allende residue (●), *a* is 3CS2; *E* trends observed in another study[27] on xenon released by stepwise heating of three Allende residues (○, ●) 4C57 (*a*), 4C56 (*b*) and 3CS59 (*c*); *F*, trends observed[15] in xenon released by stepwise heating of a Murchison residue (●) (*a*, ICIO). Other symbols are defined in Figs 1 and 2. All error limits greater than the size of the symbols are shown, except for the two temperature fractions of Murchison (Fig. 3*F*) with low values of ^{124}Xe/^{130}Xe. Error limits were not reported[15] on the latter. △, Air; ⬡, AVCC.

used in the precipitation of osmium and ruthenium. Attempts to measure the isotopic compositions of osmium and ruthenium were abandoned after our results showed that these elements are highly enriched on the residues, apparently as a result of their insolubility in the etching acids[46], and display no large isotopic anomalies.

A Ge(Li) detector and a thin-crystal, intrinsic Ge detector were used with a 1,024-channel analyser to determine the activity of each tellurium isotope relative to the activity of ^{129}Te in the samples and in the monitors. The latter were prepared from purified TeO_2 (lot 766061, Fisher Scientific). Possible interferences which might produce $^{i+1}$Te from neutron-induced reactions on other elements in the samples are (1) neutron-induced fission of uranium to produce ^{131}Te and ^{129}Te, (2) the (n, p) reaction on iodine and the (n, α) reactions on xenon, and (3) the (n, γ) reaction on ^{124}Sn followed by two successive β decays. Our results indicate a ^{128}Te concentration of 16 p.p.m. in the carbon and 21 p.p.m. in spinels, corresponding to total tellurium content of 51 and 67 p.p.m., respectively, if all other isotopes were present in the proportion observed for terrestrial tellurium. The activity of fission-produced ^{133}I and ^{132}Te in each residue and in the uranium monitor indicate that interference from neutron-induced fission of uranium produces 1.1% of the ^{131}Te activity and 0.5% of the ^{129}Te activity in the irradiated carbon, and fission interference produces 0.9% of the ^{131}Te and 0.4% of ^{129}Te activities in the irradiated spinels. The results of our tellurium and xenon analyses (Table 1) yield atomic ratios of Te/Xe ≈ 5×10^4 in carbon and Te/Xe ≈ 1.5×10^5 in spinels. From the amounts of radiogenic ^{129}Xe in these residues (Table 1) and an assumed value of $^{129}I/^{127}I = 10^{-4}$ for trapped iodine[47], we calculate atomic ratios of Te/I ≈ 130 in carbon and Te/I ≈ 480 in the spinels. The ^{127}I (n, p) reaction and the $^{124-134}$Xe (n, α) reactions have Q values of about 0.1 MeV and 2.5–6.3 MeV, and they are inhibited by Coulomb barriers of about 8 MeV and 14 MeV, respectively. Thus, each of the interference reactions listed under item (2) requires neutron energies above 8 MeV. All of the relevant cross-sections for (n, p) and (n, α) reactions are not known, but typically these are only a few millibarns for 14 MeV neutrons on target elements with $Z \approx 50$–60 (ref. 48). In the position where our samples were irradiated, the flux of neutrons above 8 MeV is about 1×10^{-3} of the thermal flux. From the atomic ratios given above and assuming cross-sections of 1,000 mb for each (n, p) and (n, α) reaction, we estimate upper limits for interference on any tellurium isotope to be 0.03% from the (n, p) reaction and 1×10^{-4}% from the most favourable (n, α) reaction. Concentrations of tin, the target element for interference from above item (3), were not measured in our Allende residues. In other Allende residues prepared in a similar manner, the highest concentration of germanium, a neighbour of tin in the Group IV A elements, is reported[46] to be 37 p.p.m. If our Residue B (carbon + spinels) contained 37 p.p.m. germanium, we calculate lower limits on the atomic ratios of Te/Sn ≫ 11 in carbon and Te/Sn ≫ 18 in the spinels, assuming a solar value for the Sn/Ge ratio[49] and assuming that the mineral fraction being considered, carbon or spinels, each contain all of the germanium of Residue B. The ^{125}Sn produced by neutron-capture on natural ^{124}Sn must undergo two β-decays before becoming an interference at ^{125}Te. An intermediate decay product, ^{125}Sb ($t_{1/2} = 2.73$ yr), acts to shield ^{125}Te from most of the ^{125}Sn decays, and we calculate an upper limit of 0.1% for the interference at ^{125}Te from the ^{125}Sn (n, γ, β, β) reaction. Interference from he (n, 2n) reaction on ${}^{i+2}$Te is expected to be negligible; for these reactions, $Q \gg 8$ MeV.

The results of counting data accumulated from 5 February 1978 to 28 May 1978 are shown in Table 3, corrected for the fission interference discussed above. It should be stressed that we measured only relative abundances of tellurium isotopes in the monitors and in the samples. The maximum variation that Smithers and Krouse[50] observed in isotope ratios in six natural tellurides and two commercial tellurium salts corresponds to a fractionation of only 0.05% per mass unit, variations which could not be detected by the techniques used in this study. We therefore assume that the tellurium in our monitors has normal values for isotope ratios, and that the activity of each isotope, ${}^{i+1}$Te, is iroportional to the abundance of the stable (n, γ) target isotope, ^{i}Te. Thus, the activities shown in Table 3 can be expressed in an analogous manner to that given for xenon isotopes in equation (1).

$$g'_{128} = ({}^{i}\mathrm{Te}/{}^{128}\mathrm{Te})_{\mathrm{sample}}/({}^{i}\mathrm{Te}/{}^{128}\mathrm{Te})_{\mathrm{standard}} \qquad (2)$$

The results of our analyses on tellurium isotopes in Allende residues are shown graphically in Fig. 4. As ^{126}Te may contain a radiogenic decay product from *in situ* decay of a precursor isotope produced in the r-process, ^{126}Sn ($t_{1/2} \sim 10^5$ yr) (ref. 51), the abundance of ^{126}Te is not used to define the general anomaly pattern of tellurium isotopes trapped in these residues. No data are available on the isotopic composition of tellurium in average carbonaceous chondrites, AVCC tellurium. Therefore, the patterns of isotopic anomalies shown in Fig. 4*A* (carbon) and Fig. 4*B* (spinels), where the isotopic ratios are normalised to the terrestrial tellurium standard, are not analogous to the patterns shown in Fig. 1 for xenon in these same residues. The xenon spectra in Fig. 1 are normalised to an AVCC xenon standard.

In spite of differences in reference standards used in Figs 1 and 4, the following observations may be significant: (1) The nonradiogenic isotopes of tellurium and xenon in Allende's carbon, as shown in Fig. 1 and Fig. 4*A*, produce remarkably similar anomaly patterns. Both elements are enriched in the heaviest isotopes, ^{130}Te and ^{136}Xe, and in the lightest isotopes, ^{120}Te and ^{124}Xe. These isotopes are produced on a very short time scale by the r and p processes in supernova explosions[31,32]. (2) The spinel-rich fraction of these minerals (Figs 1 and 4*B*) is enriched in the intermediate mass isotopes of each element, ^{130}Xe and ^{124}Te. These isotopes are generated over long periods of stellar evolution by the s process[14,31]. (3) The existence of isotopically anomalous tellurium in Allende cannot be explained by a single AVCC-type tellurium, since the isotopic spectrum of tellurium in carbon is obviously different from that in the spinels. (4) The spinel-rich fraction of Allende residues is enriched in ^{130}Te (Fig. 4*B*) but not in ^{136}Xe (Fig. 1). This apparent discrepancy between the abundances of r products in tellurium and xenon may indicate that etching the Allende residues has separated the r and p products of tellurium, just as stepwise gas extraction separated the r and p products of xenon (Fig. 3). Or this may be due to differences in the reference standards for the xenon and tellurium spectra, that is, it is possible that AVCC tellurium is systematically enriched in ^{130}Te relative to terrestrial tellurium.

Nucleogenetic heterogeneities in element abundances

In using the results of this study to evaluate the merits of models suggested for element synthesis shortly before condensation[17–19], it is imperative to consider the constraints imposed by earlier analyses[26] on noble gases in Allende's residues. These measurements revealed elemental abundances of helium and neon which correlate linearly with abundances of the r products in xenon[17–19], as shown in Fig. 5, and the correlations yield zero values of helium and neon at ^{136}Xe/^{132}Xe = 0.32±0.01. This value of ^{136}Xe/^{132}Xe contains the range of isotopic ratios for planetary xenon in air, carbonaceous chondrites and ureilites. It is now widely accepted that variations of the ^{136}Xe/^{132}Xe ratio in carbonaceous chondrites may be nucleogenetic[1,5,9,12–23,33], and that stellar evolution selectively depletes low-Z elements from the hotter stellar interior[31]. It can be concluded that normal, planetary xenon was synthesised in the hot stellar interior and that the inertness of noble gases made it possible for these elements to preserve a record of both elemental and isotopic heterogeneities which existed before condensation. Most other elements formed compounds which were chemically segregated into different minerals of planetary solids. Small variations in the isotopic compositions of normal planetary xenon, as seen in bulk ureilites[53,54], carbonaceous chondrites[55], and air[56], are identified in Fig. 5 at values of ^{136}Xe/^{132}Xe = 0.313, 0.322 and 0.330, respectively. Correlation coefficients between the elemental and isotopic ratios shown in Fig. 5 are between 99.2% and 99.9%. It has been reported[26] that a plot of ^{124}Xe/^{130}Xe against ^{136}Xe/^{130}Xe for xenon in these same residues rave a correlation coefficient of 98%.

We did not determine helium and neon in our study but have used the trends shown in Fig. 5 in interpreting our results, since the results of other studies on noble gases in residues of Allende[27,33,57] and Murchison[58] confirm these correlations.

Table 3 Ratios of γ-ray intensities detected from activated tellurium isotopes

Isotopes	Carbon-terrestrial	Spinels terrestrial	Carbon spinels	γ-Energies detected (MeV)	Half-life observed
^{121}Te	106.9±2.6	102.3±1.9	105.4±1.4	0.573	20–30 d
^{123}Te	104.3±2.5	100.1±2.4	103.7±2.2	0.159	120 d
^{125}Te	97.6±0.5	102.4±0.6	95.4±0.5	0.035*	58 d
^{127}Te	105.1±4.1	100.4±3.9	103.4±3.6	0.418	110 d
^{129}Te	≡100.0	≡100.0	≡100.0	0.696, 0.027*	34 d
^{131}Te	111.4±4.0	117.5±3.4	94.8±1.7	0.775, 0.850	30 h

* Counted with intrinsic Ge detector

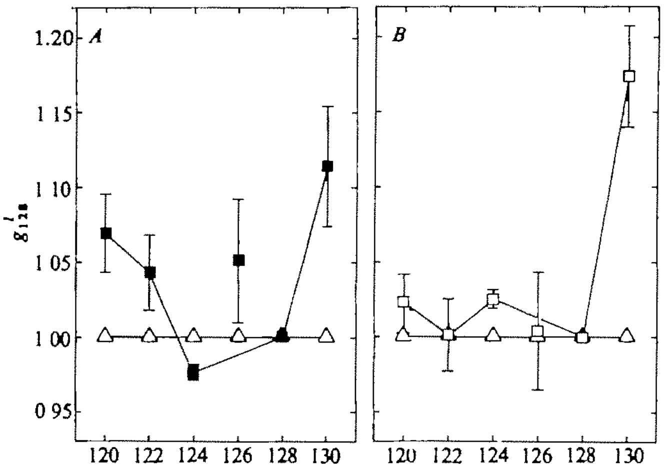

Fig. 4 Isotopic compositions of tellurium in Allende's carbon (■) and spinels (□) normalised to that in the tellurium monitor (△) in the manner indicated by equation (2). Since ^{126}Te may contain the *in situ* decay product of ^{126}Sn, it is not used to define the isotopic abundance pattern of trapped tellurium. *A*, The anomaly pattern of tellurium isotopes in carbon resembles that observed for xenon isotopes (Fig. 1) in carbon; *B*, the anomaly pattern of tellurium isotopes in spinels reveals an enrichment of the s product, ^{124}Te, and the r product, ^{130}Te.

These correlations are also seen in bulk noble gases of meteorites. Most carbonaceous chondrites contain helium and neon, and stepwise heating[1] or chemical etching experiments[26] on carbonaceous chondrites reveal the presence of an anomalous xenon component with ^{136}Xe/^{132}Xe>0.32. However, Marti and Wilkening[59] report that the stepwise release of gases from the Kenna ureilite revealed the presence of a single xenon component with ^{136}Xe/^{132}Xe$=0.312$, but they found no evidence of trapped helium or neon although the ureilite contained large amounts of heavy noble gases.

Conclusions

The presence of isotopically anomalous tellurium in Allende provides additional evidence for the suggestion that isotopically anomalous components in xenon in carbonaceous chondrites are nucleogenetic[1,5,9,12–23,33] rather than fission products[2,26,27,46,58]. The following observations are difficult to explain by alien grains carrying nucleosynthetic products from supernovae[12,13] or red giant stars[14,15]: (1) The carbon grains have a normal value of ^{12}C/^{13}C (ref. 30), (2) the carbon contains anomalous xenon, krypton and tellurium and normal helium and neon[29], (3) isotopic ratios of xenon enriched in s products lie on the correlation line defined by isotopic ratios of xenon enriched in r and p products (Fig. 3*A*, *C*), and (4) there was no helium or neon in the primitive noble gas component which contained normal, planetary xenon (Fig. 5 and ref. 59). Trapping of alien nucleosynthetic elements from a nearby supernova[1,16] onto a matrix of isotopically normal material can explain observations (1) and (2), but not observations (3) and (4). The observation that r and p products from the supernova event are not mixed (Fig. 2, Fig. 3*B*, *D–F*, Fig. 4) also requires that the injected material remain heterogeneous as it was transferred from the supernova to the solar system. Even if this occurred, it seems highly unlikely that the abundance pattern of xenon isotopes from supernovae[1,16] and red giant stars[14,15] would be mirror images and that these two alien forms of xenon would be fortuitously trapped in meteorites in which the bulk xenon isotopes display an intermediate abundance pattern (Fig. 1).

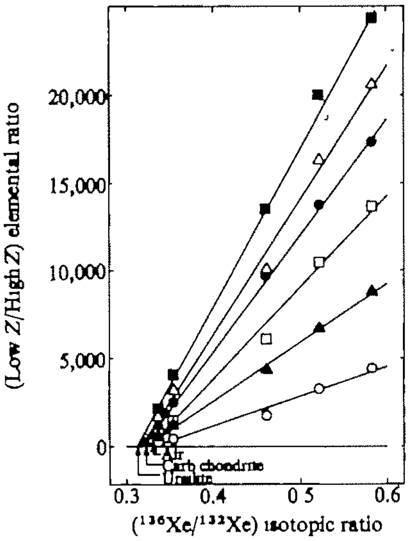

Fig. 5 Correlations between values of the isotopic ratio, ^{136}Xe/^{132}Xe, and elemental ratios of (low-*Z*/high-*Z*) noble gases in residues of Allende[26]. Measured values of elemental ratios are multiplied by constants so that all six ratios can be shown together. Values of (^{20}Ne/^{132}Xe)×60 are depicted by (■), (^{4}He/^{132}Xe)×0.25 by (△), (^{20}Ne/^{36}Ar)×4,000 by (●), (^{20}Ne/^{84}Kr)×25 by (□), (^{4}He/^{36}Ar)×10 by (▲), and (^{4}He/^{84}Kr)×0.04 by (○). The correlations yield zero values for the elemental abundances of helium and neon at a value of ^{136}Xe/^{132}Xe$=0.32\pm0.01$. This corresponds to the ^{136}Xe/^{132}Xe ratio of bulk xenon in air, carbonaceous chondrites, and ureilites, as shown at ^{136}Xe/^{132}Xe$=$ 0.330, 0.322 and 0.313, respectively.

The isotopically anomalous components of tellurium, xenon and krypton observed in this study can be understood as remnants of the different nucleosynthesis processes which collectively produced the chemical elements of the Solar System. The opposite anomaly patterns of xenon (Fig. 1), the incompletely mixed r and p products in xenon (Fig. 2, Fig. 3) and in tellurium (Fig. 4), the association of isotopically anomalous high-*Z* elements with isotopically normal low-*Z* elements, and the absence of helium and neon in the noble gas component with normal, planetary xenon (Fig. 5, ref. 59) are consistent with the suggestion that our Solar System formed directly from the debris of a single supernova[17–19].

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DNA sequences and structural homologies of the replication origins of lambdoid bacteriophages

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*The DNA sequences for the origins of replication of the lambdoid bacteriophages φ80, 434, φ21, and λ*imm*21 (identical to φ21) have been determined and compared to the λ structure. Two presumptive elaborate binding sites for two initiator proteins have been identified in their outer sections, while a replicational primer start site seems to be located in their centres.*

BACTERIOPHAGE λ initiates DNA replication bidirectionally from a unique site on its circularised genome. This site has been located at approximately 80% of the linear DNA molecule measured from the left end[1,2]. A group of *cis*-dominant, replication-defective λ phages (λ *ori*$^-$) has been isolated[3,4], and four such mutations have now been characterised by DNA sequence analysis[5,6]. All are located within the λ *O* gene[7], immediately to the left of an *Eco*RI restriction site at 81% λ (ref. 8). While the segment of λ DNA covered by the *ori*$^-$ mutants (comprising 63 base pairs, ref. 5) gives a lower limit for the λ origin sequence, a 202 base pair upper, functional limit has been observed in hybrid plasmid cloning studies[9].

The lambdoid phages 434, φ21, and φ80 are genetically very similar, and can exchange functionally corresponding though not necessarily identical (or heteroduplexing) segments of their genomes with λ or with each other[10–12]. Heteroduplexing DNA segments can also be observed in electron microscope analysis of pairs of lambdoid phage DNAs[13–15]. In particular, the regions surrounding the origins of replication, although located at somewhat different positions within the various phage DNAs, form heteroduplexes among the subgroup λ, 434 and φ21, whereas the corresponding region of φ80 DNA seems to be different, and heteroduplexes with λ only in a right-hand section of that region. Genetic observations[16] also indicate exchangeability of the analogous gene *O* products among λ, 434 and φ21, while the φ80 *O* function is not exchangeable with any of the others. The φ80 *O* protein is also different in size[17]. The products of the various *P* genes located to the right of the *O* genes can, however, be exchanged among all members of this group (even if only asymmetrically in one case[17]), although functional differences can still be detected[18].

While the origin of replication constitutes at least part of the replicator structure as proposed by the replicon hypothesis[19], the phage coded proteins O and P seem to fulfill the role of specific initiator proteins also predicted by this model. And the specific binding of the phage's own initiator protein(s) to its replicator structure seems to determine at least in part the individuality of these phage replicons, as being distinct from the cellular genome. In addition, besides the requirement for a supercoiled, circular DNA substrate[20], initiation of λ replication has been shown to depend on local transcription at or near the origin of replication (transcriptional activation)[21–24]. When initiated, λ DNA synthesis requires most of the proteins that are essential parts of the *Escherichia coli* replication machinery, including *dna*B, *dna*G, and *dna*E (polymerase III)[18].

Detection (in a colE1–λ hybrid plasmid system) of a second, unconnected replicator element located within the *c*II gene has led us to redefine the λ (lambdoid) origin of replication as a signal structure that constitutes (only) the start site for a replicational primer synthesis, most probably catalysed by *dna*G coded primase[9]. DNA strand separation is a prerequisite for daughter strand DNA (or RNA) synthesis, and by definition the origin of replication in a preceding step must also be the origin of strand separation during initiation; strand separation also seems to be required for the action of primase[25]. We assume, therefore, that facilitation of local DNA strand separation, and hence exposure of a promoter-analogous primase binding site[26,27], will be a basic property of the origin of replication. In addition, it may possibly also be involved in attachment of the DNA replicon to a structurally organised replication site (replisome), and in protection of the nascent replicational primer 5′-end (for discussion see ref. 28).

To look more closely at the lambdoid origin of replication we have sequenced the 434, φ21 and φ80 origin segments and compared them to λ (ref. 6). On the basis of this analysis, the λ (lambdoid) origin of replication can be deduced from structural considerations to consist of 156 base pairs, extending in both directions from its 63 base pair minimum, and included within the 202 base pair maximum size as mentioned above.

Aligning lambdoid origin DNA sequences in parallel

The various origins of replication were sequenced using the dimethylsulphate/hydrazine technique[29] (Figs 1 and 2). The 434 and φ21 sequences are almost identical with λ, but the φ80 DNA sequence differs from the others for most of its length. All four DNA sequences, however, are identical or at least nearly identical (see boxes in Fig. 3) in several stretches within the functional limits of the λ (φ21) origin[9]. The largest of these stretches is 31 base pairs long with only two internal λ/φ80 non-homologies (one is also found in φ21 DNA), and includes the *Eco*RI cleavage site common to all of them. The coding frame for this part of the *O* gene sequence also seems to be the same. With the φ80 DNA sequence clearly diverging from the other lambdoid DNA sequences to the left of position λ:1,099/φ80:1,100 and to the right of position λ:1,169/φ80:1,170, we regard the 31 base pair identical stretch to be a fixed point around which all the origin sequences can be aligned both structurally and functionally.

Against this background, λ/φ80 DNA homologies provide positive evidence for functionally important segments within the origin of replication structure, which can be cross-checked against defective λ *ori*$^-$ mutant[5,6] or other partial deletion[9] sequences. The sequence deviations within the λ/434/φ21 group are considered to point to positions of minor functional importance. Although some of the sequence deviations between φ80 DNA and the other lambdoid phages may similarly point to positions of minor importance, others should indicate specific alterations to serve analogous, but phage-specific functions.

When the DNA sequences of the lambdoid origins of replication are aligned in parallel they display a surprisingly complex, but analogous order of symmetry elements. Taking other characteristic features into account, three distinct regions with very little overlap (Fig. 3) can be identified. Besides these three sections there are two short external tail segments, which differ

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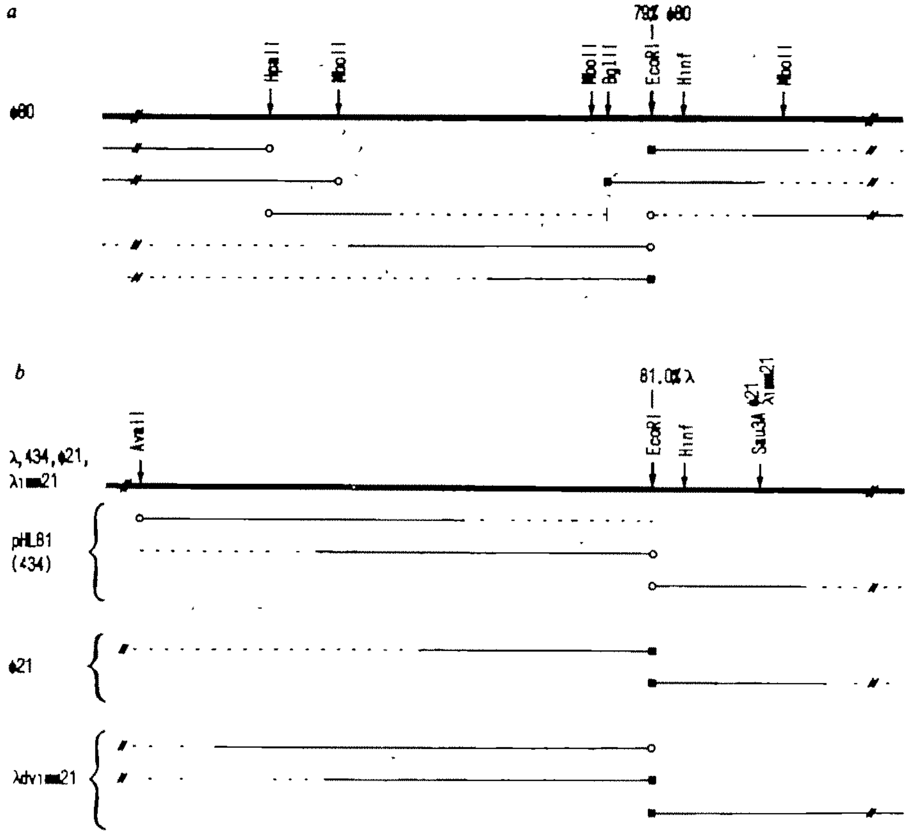

Fig. 1 Physical maps and schematic protocols for the sequence analysis of the ori regions of lambdoid phage DNAs. *a*, Sequence analysis of the *ori*-region of phage φ80 DNA. *b*, Sequence analysis of the *ori*-region of other lambdoid phages. Sequencing was performed with plasmid pHL81 DNA[9], carrying the *ori*-region of phage 434, φ21 DNA, and plasmid λdv*imm*21AB5 DNA[36], carrying the *ori*-region of phage λ *imm*21. Double-stranded DNAs are indicated by double lines, with positions of restriction endonuclease cleavage sites indicated by vertical arrows. Single lines drawn below represent single strands of restriction fragments used for sequencing, each carrying one end labelled. An open circle indicates a labelled 5′-end, and a line extending to the right of the circle, for example, represents the *l*-strand of a fragment used for sequence determination, in 5′- to 3′-direction. A 3′-end labelled is marked by a square. The solid part of each line indicates the portion of the fragment for which unambiguous sequence information could be obtained. A vertical bar shows the secondary restriction site used for cleavage of one of the labelled fragments, while other secondary cleavage sites used lie outside of the region shown. Terminally labelled single strands if obtained by strand separation (for example, the *Ava*II–*Eco*R1 fragment of pHL81–DNA) are not explicitly designated in the drawing

in sequence for λ and φ80 and which do not include such structural symmetries; they probably do not belong to the origin of replication proper. An 8 out of 9 base pair identical sequence in the left tail segment may be coding for an essential element in the O protein structure, because it is read in the same frame: Ser-Glu/Ala-Trp. Only one half of an (additional) inverted repeat sequence is retained within the λ right tail sequence, the second half is located beyond the 202 base pair maximal origin boundaries—this may even be used as an argument against the remaining tail segment's being of any importance.

Conserved length of unique composition in the central region

The central (B) section (λ 1,099–1,135) of the lambdoid origins of replication consists of a stretch of DNA sequence which is uniquely rich in A in the *l*-strand, and in T in the *r*-strand, including an uninterrupted sequence of 15A + 3G in the λ origin DNA, as first observed by F. Blattner and colleagues[5]. While this general characteristic is clearly seen in the φ80 origin DNA, the φ80 sequence is of slightly less extreme composition. There appear to be three segments of identical or nearly identical DNA sequences in a section B λ/φ80 comparison (see boxes in Fig. 3) which may be indicative of their functional importance, but the intervening groups of nucleotides are also rather similar and are identical in length, except for the deletion of one base pair in the φ80 DNA (corresponding to λ : 1,136) at the junction between the origin B and C segments.

In contrast to the flanking regions A and C, the central section of the origin of replication does not contain any inverted repeat sequences with the potential for being folded into single stranded hairpin structures; instead it very characteristically does contain DNA mirror sequences (true palindromes). One of them, in the λ-DNA group, extends over 28 base pairs with a single (λ, φ21) deviation only (considerably disrupted, however, in 434 DNA). It is immediately followed to the left by a second one of 12 base pairs (1 deviation). As a result of several nucleotide exchanges, the very long DNA mirror sequence is not prominent in the φ80 origin of replication DNA; it is in part 'suppressed' by another, perfect 15 base pair mirror sequence, which asymmetrically overlaps with the first. Since an asymmetric DNA composition of the kind observed in section B would contribute to forming such mirror DNA sequences, they can still be maintained to be accidental, and their part in the overall origin function—if any—remains uncertain.

Segments of double-stranded DNA sequence of the composition observed for section B should have very low melting points as a result of their high A–T contents. According to reference data[30] the T_m can be calculated for the λ and φ80 section B DNAs to be about 78.5 °C and 79 °C, respectively (in 1 × SSC; 12 °C below λ DNA average). Specialised sections of such sequence, when their strands become separated in an RNA polymerase catalysed transcriptional activation reaction (see below), should transiently be converted into a rigid, open helical conformation, due to the purine stacking interactions in their separated *l*-strands.

Specific sequences exposed in (protein coated) single-stranded templates are known to be required for binding and initiating primer RNA synthesis by *dna*G coded primase, and the same sequences, if present in double-stranded DNA, will not be recognised by this enzyme[25]. In a λ–colE1 hybrid plasmid genetic analysis a replicational primer synthesis has been observed to be initiated within the origin of replication boundaries in an asymmetric, only right to left orientation, and to be insensitive to an inserted transcriptional terminator[9]. λ replicational primer synthesis, therefore, seems to be catalysed not by RNA polymerase, but most probably by primase, already known to be involved in initiation of DNA replication in other systems[23,24]. The central section of the origin, which coincidentally has a very low melting point, and contains the DNA sequences analogous to the G4 origin[26,27], also in a right to left orientation, seems, therefore, to be the most obvious choice for an initiation site for the λ primer synthesis observed *in vivo*. This is confirmed by the fact that the RNA polymerase is not bound to various φ80 DNA fragments carrying the origin of replication, in a filter binding assay (in 125 mM KCl, 10 mm $MgCl_2$, 20 mM Tris HCl (*p*H 8.0) at 37 °C, with competition by poly(rI) (ref. 31, results not shown). The overall rifampicin sensitivity of λ replication[18,23,24,32] may then have to be attributed to the preceding RNA polymerase catalysed transcriptional activation of the origin structure, usually in a left to right direction across its location.

Asymmetric oligo dA : dT segments have also been observed in other, unrelated double-stranded origins of replication[33], and A-rich sequences for single-stranded phage DNA origins[26,27]. Comparison between their sequences may lead to the identification of a promoter-equivalent primase signal structure.

Two λ origin-defective mutants, in which different parts of section B are deleted (λ *r*99 : 1,098–1,109; λ *r*96 : 1,116–1,130) have been sequenced to date[5]. Both of them show a completely defective mutant character.

Two overlapping DNA hairpin loops in the right-hand section

The origin section C shows two different characteristic organisational features which are both contained in the same stretch of the DNA. One is the exceptional, 31 base pair sequence, which is identical (with two exceptions) for all four phage DNAs. Second, this section contains two almost perfect inverted repeat sequences which overlap asymmetrically. On DNA strand separation the inverted repeat sequences could give rise to two different kinds of DNA hairpin structures (see Fig. 4). These 33 base pairs at the origin of replication (λ : 1,136–ϕ80 : 1,168) therefore apparently have to fulfill triple functions. First they code for 10 amino acids of the gene *O* product, and second and third, they act as parts of the longer and the shorter DNA hairpin loops. DNA segments with multiple functions have also in other cases been shown to be highly conserved throughout evolution[34].

Because the section C sequences are almost identical in all four phages, any candidate initiator protein(s) binding to them must also be identical or at least exchangeable among all four phages. These should, therefore, be the phage P proteins, or one of the host initiators, such as the *dna*B protein (or a complex of both).

If the λ origin of replication is cut within section C at the *Eco*RI restriction site and is fused onto various foreign, *Eco*RI terminated fragments, the overall replicational activity (in the presence of the O protein) will drop to 3–5% of its original value, as observed in the λ–colE1 hybrid plasmid system[9]. This indicates that section C is an integral part of the overall λ replicator structure. The functional leakiness of the deletion variants may be due to either the activity of the remaining fraction of section C DNA, or else may indicate a potentiating rather than a basic function for section C.

DNA cloverleaf structure in the left-hand section

Section A of the lambdoid origins of replication displays the largest number of symmetry elements (Fig. 3). These are organised in the form of direct repeats, which consist internally of an inverted repeat substructure. Also, the direct repeats are very regularly spaced within the section A sequence, and therefore higher orders of symmetry involving several such elements can be considered. All such considerations of structural symmetry originate from the multiple repetitions of a basic sequence 'theme' (boxed in Fig. 3), alternating in direct and inverted orientations. While the λ and ϕ80 linear section A DNA sequences differ from each other, they are organised in a clearly analogous manner with the total and very complex arrays of symmetry elements present in nearly identical arrangements. The sequence 'themes' basic to all of these analogous configurations, however, are different, even in length: λ, 434, ϕ21: GAGGGAT; ϕ80: CGGGAA.

If DNA strand separation is accepted as likely to occur at the origin of DNA replication, any one of the section A inverted sequence repetitions could be folded into a single-stranded DNA hairpin structure, and therefore several 'activated' structural arrangements are possible for a segment of such complex organisation. A collection of potential secondary structures is represented in essence by the arrows drawn in Fig. 3. However, the only one of these potential activated structures which involves all the symmetry elements at the same time, and also makes use of their regular spacing along the DNA of this section, is a DNA cloverleaf arrangement (Fig. 5). Identical superstructures can be drawn for ϕ80 and for λ, in spite of the differences between their individual elements. When folded into such higher order of symmetry arrays, the repeated sequences of either six or seven nucleotides form the individual branches and stems of the cloverleaf structures. Quite consistently, it is also

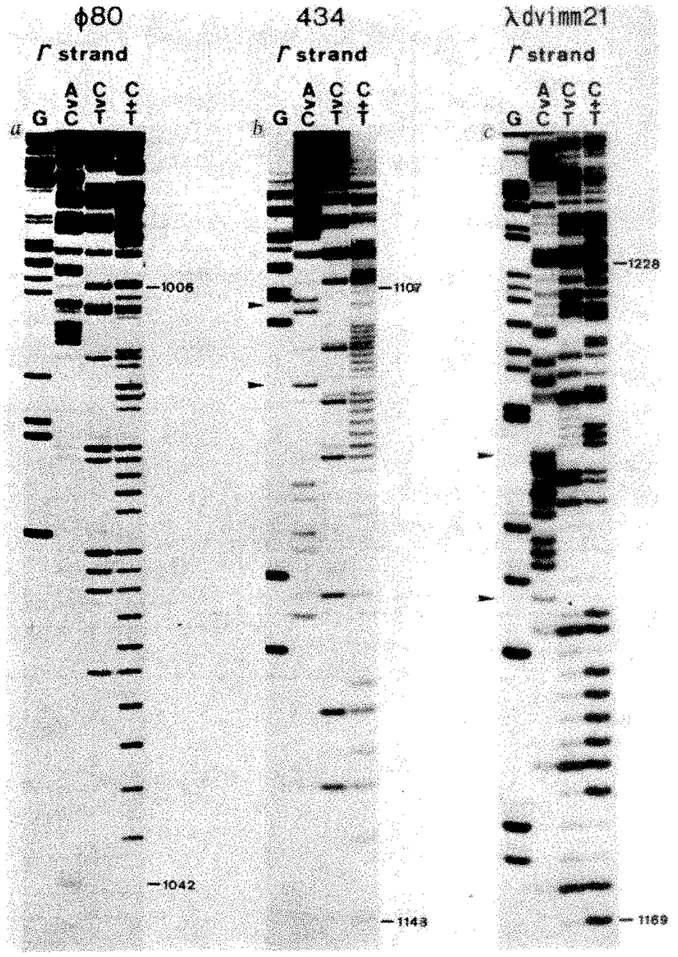

Fig. 2 Autoradiographic patterns of DNA sequencing gels after electrophoretic separation of the chemical degradation products of various origin DNA restriction fragments. The lanes designated by G, A > C, C > T, and C + T display the products of the corresponding reactions. DNA fragments derived from the various origin of replication regions as observed in preceding restriction mapping have been terminally labelled with ^{32}P-phosphate at either their 5′-ends by using polynucleotide kinase and [γ-^{32}P] ATP (ref. 29) (*a*–*b*) or at their 3′-ends by DNA polymerase I and [α-^{32}P]dATP (refs 37, 38)(*c*). For DNA sequencing the dimethylsulphate/hydrazine technique[29] has been used throughout this work. The numbers to the right of each gel refer to the corresponding nucleotide positions in the *l*-strand DNA sequences shown in Fig. 3, while arrows to the left (in *b* and *c*) indicate some of the sequence deviations in the *ori* regions of pHL81(that is, phage 434) DNA (*b*), and λdv*imm*21AB5 DNA (*c*), with respect to the homologous λ DNA sequence[6]. The deviations in the *ori* region of λdv*imm*21AB5 DNA could also be observed in ϕ21 DNA (compare legend to Fig. 3). *a*, Sequence pattern obtained from the 5′-labelled *Mbo*II fragment 338–1,046 derived from ϕ80 DNA, which was recut by *Hae*III at position 892. *b*, Sequence pattern from the 5′-labelled *Eco*RI fragment of pHL81 DNA (434 DNA) 624–1,146, recut by *Bgl*II at position 792. *c*, Sequence pattern from the *Eco*RI-linearised, and at position 1,146 3′-labelled λdv*imm*21AB5 DNA, recut by *Taq*I at position 1,466. The degradation products were loaded on 40 × 22 × 0.2 cm gels (*a* and *b*) or 90 × 16 × 0.2 cm gels (*c*) of 20% polyacrylamide/7 M urea, and were electrophoresed for 6 h at 1,000 V (*a* and *b*), or for 24 h at 2,000 V (*c*).

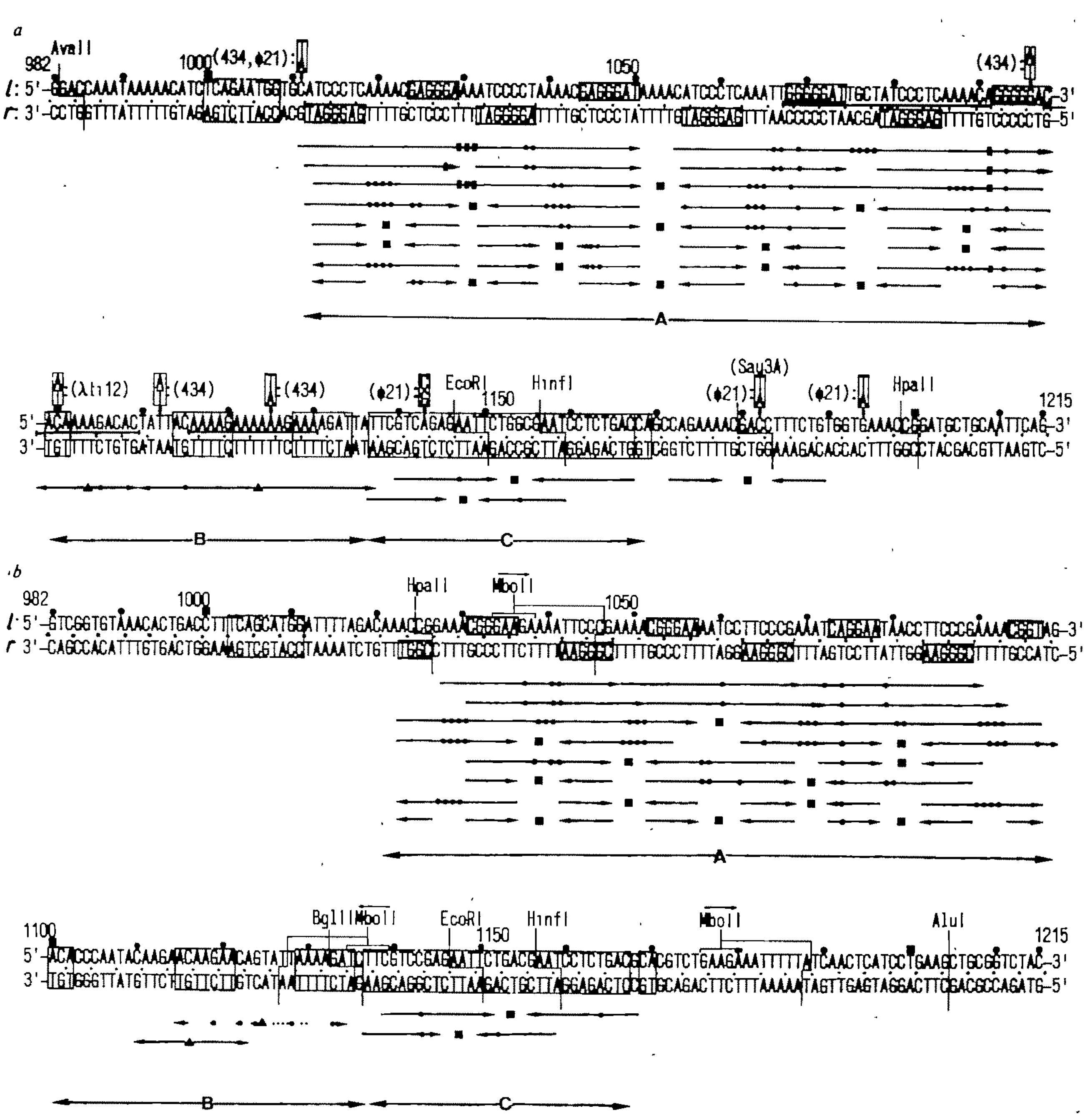

Fig. 3 DNA sequences of the λ (*a*) and φ80 (*b*) origins of replication in the λ (φ21) minimisation boundaries (982–1,182) (ref 9). The nucleotide positions in all four phage DNAs are normalised, for 1,146 to indicate the first nucleotide (G) of the common *Eco*RI cleavage site located within their origin of replication sequences This is equivalent to position 1 indicating the first nucleotide transcribed into the p_R promoted mRNA, in λ DNA[37]. Nucleotide exchanges observed for the homologous 434 and φ21 DNA in an otherwise identical sequence are indicated above the λ sequence, the position 1,100 transversion determined for the λ *ti*12 mutation[6] has also been included. Selected restriction endonuclease cleavage sites are referred to in their standard abbreviations. The *O* gene reading frames are marked by dots between the two lines showing the DNA sequences for both complementary strands, and the nucleotide segments deleted in three λ *ori*⁻ deletion mutants[3] are also indicated (———)· reading from left to right, λ *r*93, λ *r*99 and λ *r*96. Double strand boxes mark segments of λ/φ80 DNA homology, single strand boxes point out multiply repeated sequence 'themes' present in the left sections The symmetry elements observed in the origin DNAs are indicated below the sequences. Direct repeats have been marked by repetitive arrows, inverted repeats by two arrows pointing towards their inversion centre (square), the arrows indicating a mirror DNA sequence have been orientated away from its centre (triangle). G · T/A · C symmetry deviations in inverted repeats have been indicated by one dot (in the *l*-strand purine location), A G/T · C mismatches by two dots (in both locations), vertical bars in *a* mark positions of nucleotides inserted or deleted over a perfect match. In the left parts, the lower-most arrangements of symmetry elements correspond to the activated DNA cloverleaf structures presented in Fig. 5 Three structurally different sections of the origin sequences as deduced from the distribution of symmetry elements are indicated at the bottom (A, B, C). In comparing the origin DNA sequences of λ, φ21 and hybrid λ *imm*21 DNAs (plasmid λdv*imm*21AB5 (ref. 36), and phage λ *imm*21 (ref. 11)) we observed that the hybrid λ *imm*21 DNA in this region consists of φ21 rather than λ DNA. This has been concluded from the presence of the exchanged φ21 nucleotides instead of the λ specific nucleotides at every divergent position analysed. 1,011, 1,143, 1,182, 1,194 (and 1,233, not shown) The nucleotide exchange C→T at position 1,182 results in an additional *Sau*3A recognition site not present in λ DNA, which has been used in our origin minimisation cloning studies[9] Therefore, the origin limits as indicated are actually those of φ21 DNA, which however should also apply to the nearly identical λ DNA Restriction analyses of φ21 and λ *imm*21/λdv*imm*21 DNAs in comparison with the λ DNA *O* and *P* gene sequences (ref. 6 and E Schwarz, unpublished) resulted in recognition of a number of restriction sites present in both φ21 and λ *imm*21 DNA, but not present in λ DNA, up to at least the centre of the *P* gene. Only to the right of the *P* gene is the λ *imm*21 DNA like λ DNA (not shown). λ *imm*21 and φ21 DNA have also been sequenced and compared to λ DNA in the *oop* and p_0 promoter regions[6], and in this segment, to the right of the 'λ *imm*21 boundary' at position 595 (ref 37) all three DNAs are identical for at least 117 base pairs From these results we wish to conclude that the recombination event leading to λ *imm*21—(ref. 11) took place near the right rather than the left end of the *oop-O-P* homologous section of λ compared with φ21 DNA. While position 595 now may have to be called a 'virtual boundary' with respect to the λ *imm*21 creation event, it can still be expected to mark the effective boundary of non-homology compared with homology in a phage λ *imm*21/λ recombination or DNA heteroduplex analysis.

the most complex cloverleaf configuration, which results in the smallest number of hairpin mismatches (Fig. 3). This is particularly obvious for the altogether smaller (77 base pair compared with 85 base pair), and most perfect $\phi 80$ DNA cloverleaf, which in all of its three hairpin branches only contains a single A · C/G · T (*l*-strand/*r*-strand) mismatch, due to an aberrant A:T instead of G:C in position 1,074.

The potential λ, 434, and $\phi 21$ DNA cloverleaf structures have their branches and stems expanded over the $\phi 80$ DNA cloverleaf size by one hairpin base pair each. Although this should add to their stability, they do include an increased number of mismatches (three within the branches, one or two within the stem, all of the G · T/A · C type, and some other deviations concerning the left branch). Some of these aberrations may result from amino acid sequence requirements in that part of the O protein structure, for which the origin DNA sequence is coding in addition to its fulfilling DNA replicator functions.

Besides yielding nearly perfect cloverleaf branches only the origin cloverleaf model also succeeds in placing all the four regular, significantly A-rich intervening sequences into a specific, symmetrical arrangement of their own ('connecting stretches', $\phi 80$: AAAA, λ : AAAAN, with few deviations only). Stacked A_4 groups in between the cloverleaf branches are supposed to aid in lining up the protein-complexed individual cloverleaf elements for tetramerisation. The three more variant intervening groups which generally consist of A-rich, pentanucleotide sequences, through cloverleaf folding become located into symmetrical positions of their own too, and form the single-stranded top loop sequences of the individual branches. Both groups are necessary parts of the overall cloverleaf structure, in addition to the cloverleaf branches proper. Obviously, the cloverleaf stems because of their external connections have to deviate somewhat from a regular cloverleaf branch configuration. They are still close enough in their structural arrangements, however, to qualify for a fourth branch of the section A DNA cloverleaves (more fully in the λ case).

An additional point favouring the hypothesis of a higher order DNA cloverleaf structure is that it can be built from small, individual hairpin loops used as building blocks, which are then converted into a large superstructure, through a secondary, symmetrical rearrangement (of protein subunits bound to them). In our model to explain the mechanism of transcriptional activation of the origin of replication we expect RNA polymerase to act as a DNA strand separation agent which is able to catalyse the formation of such hairpin loops within the non-coding strand, while both strands during transcription are being separated internally in the enzyme molecule[28]. Only rather short DNA hairpin loops, however, are likely to be accommodated by the enzyme molecule in such a mechanism, and activated origin structures using larger-sized individual DNA-hairpin configurations[5] (which in the middle also would contain rather weak sections, see Fig. 3) seem to be disfavoured in this kind of an activation reaction, while a stepwise formation of individually activated cloverleaf branches would be possible.

We interpret an activated section A origin cloverleaf structure to represent the single-stranded (tetramer) DNA binding site for one of the proteins active in initiation of phage DNA replication. As judged from their analogous, but individual DNA structural designs, the initiator proteins binding to the $\phi 80$ or λ DNA cloverleaves should also be analogous, but different and non-exchangeable between the two phages (exchangeable, however, within the group of λ, 434, and $\phi 21$). The obvious choices for proteins of such specificity have to be the O proteins of all these four phages, or more precisely their N-terminal domains[17]. The design of the DNA cloverleaf structures seems to predict their binding of four identical O protein subunits, each of which may capture one of the RNA polymerase activated cloverleaf branches (as indicated in Fig. 5 in a rather general way). The DNA bound protein tetramer in turn is expected to aid in stabilising the overall DNA–protein conformation through protein–protein interactions. With an activation complex (sections A–C) first installed in the non-coding, A-rich *l*-strand, the complementary *r*-strand would stay single-stranded, and therefore would have a chance of also being converted into protein-complexed secondary structures, even without a special activation mechanism of its own. While a cloverleaf activated state structure of the origin section A DNA (on its own) would be clearly less stable than its double-stranded ground-state conformation, the energy to be contributed by a cloverleaf DNA–initiator protein binding reaction, in order to compensate for the formation of single-stranded sections within this structure, has been calculated to amount to not inaccessible values for the protein subunit–cloverleaf branch interaction compared with other protein–DNA binding reactions.

One of the origin defective mutants, λ *r*93, has been shown to delete 24 base pairs (1,068–1,091) of the section A DNA (ref. 5), which if projected upon the λ cloverleaf model would correlate with exactly its right branch and lower right connecting stretch removed. The observation of a slight degree of replicational leakiness for λ *r*93 (ref. 4), conceivably might not be in disagreement with a four-branch cloverleaf model, assuming a very low rate of activity for a crippled, strained three-branch cloverleaf that might be formed in that λ *ori*⁻ mutant. The

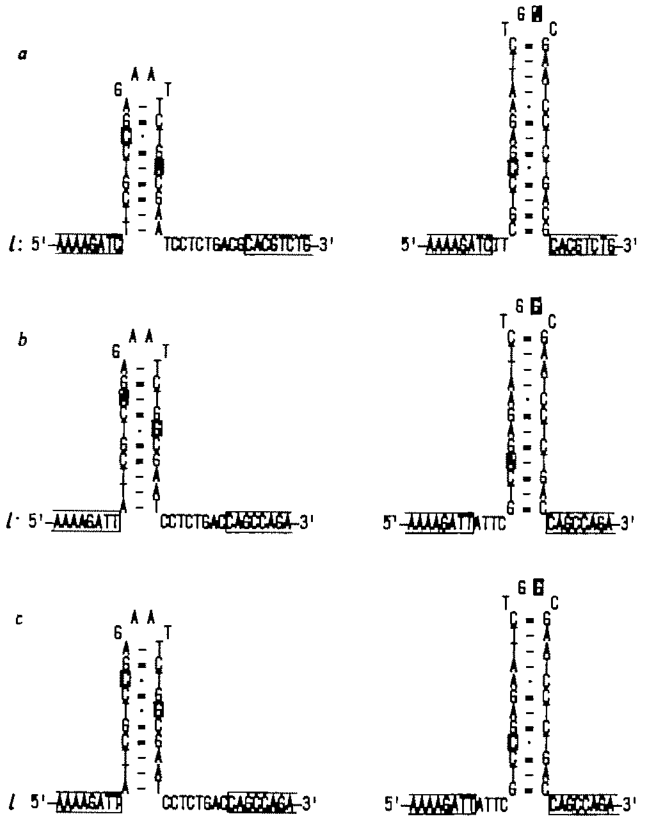

Fig. 4 Potential *l*-strand hairpin loop structures observed in the origin section C DNA sequences of $\phi 80$ (*a*), λ; 434 (*b*) and $\phi 21$ (*c*). The smaller section C DNA hairpin loops (left) are asymmetrically overlapping with the larger section C hairpin loops (right). Left and right external boxes indicate the boundaries of section B, and right tail neighbouring DNA segments, respectively; internal boxes mark the only two positions of single nucleotide exchanges among the various phage DNAs. The left end of the smaller hairpin loop coincides exactly ($\phi 80$: 1,137) or nearly exactly (λ : 1,136; the corresponding base pair, however, is deleted in $\phi 80$) with the left end of the common, identical 31 base pair segment, while the right end of the larger hairpin loop again coincides exactly (λ. 1,167) or nearly exactly ($\phi 80$·1,168) with the right end of the 31 base pair segment. We regard a structurally arranged potential for two rather than one, asymmetrically overlapping, both nearly perfect DNA hairpin loops (within a sequence also coding for a protein), and coincident with a stable, landmark section of general lambdoid origin homology, to be highly significant. And since both the DNA hairpin structures therefore may be of functional importance for the origin DNA C section, we are led to assume that this part of the structure may be shuttling between two different conformations of an activated DNA single-stranded state (and the ground state of double-stranded DNA structure). It has been possible to isolate an *SrI*⁰ mutation for the *Eco*RI recognition sequence at 81.0% (ref. 39); the nucleotide exchange(s) involved are, however, not yet known

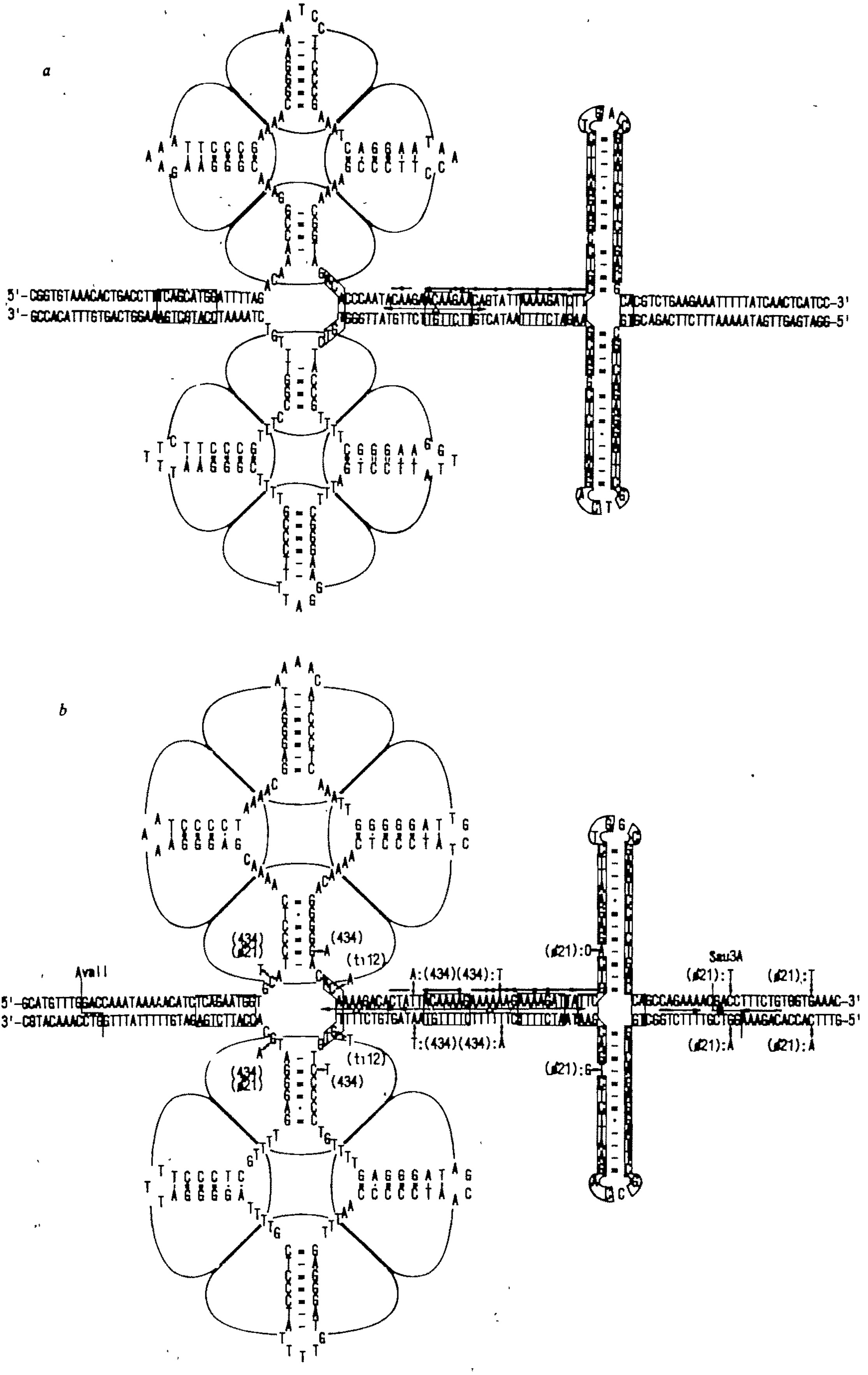
a
5'-CGGTGTAAACACTGACCTTTCAGCATGGATTTTAG
3'-GCCACATTTGTGACTGGAAAGTCGTACCTAAAATC
ACCCAATACAAGAACAAGAACAGTATTAAAAGATCTT
TGGGTTATGTTCTTGTTCTTGTCATAATTTTCTAGAA
CACGTCTGAAGAAATTTTTATCAACTCATCC-3'
GTGCAGACTTCTTTAAAAATAGTTGAGTAGG-5'
b
AvaII
5'-GCATGTTTGGACCAAATAAAAACATCTCAGAATGGT
3'-CGTACAAACCTGGTTTATTTTTGTAGAGTCTTACCA
AAAAGACACTATTTCAAAAGAAAAAAGAAAAGATTTATTC
TTTTCTGTGATAAAGTTTTCTTTTTTCTTTTCTAAATAAG
CAGCCAGAAAACGACCTTTCTGTGGTGAAAC-3'
GTCGGTCTTTTGCTGGAAAGACACCACTTTG-5'
(434)
(φ21)
(t112)
A:(434)(434):T
T:(434)(434):A
(φ21):C
(φ21):G
Sau3A
(φ21):T
(φ21):A

Fig. 5 Model proposed for the activated state structures of the ϕ80 (*a*), and λ, 434 and ϕ21 (*b*) origin of replication DNAs Only the restriction cleavage sites limiting the λ (ϕ21) origin in its maximum functional size are indicated; for inverted repeat and DNA mirror sequence symbols see Fig. 3 Segments of ϕ80/λ DNA homology are marked by boxes, segments of ϕ80/G4 and of λ/G4 DNA homologies in the central origin sections are indicated by lines above the *l*-strand sequences, with dots pointing out mismatch positions (G4, 170–172, 175–180, and 183–202 (ref. 26)). Four subunits of the (O) proteins supposed to be binding to the origin cloverleaf DNA structures have been indicated in a rather general way. Mismatches in the section A cloverleaf branches, and in the section C (larger) hairpin stems are marked by dots.

analysis of a λ *r*93 pseudorevertant DNA has shown the deleted section A sequence not to be re-installed[35], and therefore proves the λ *r*93 defect not to be due to the loss of the indicated segments as such, but to the disruption of an origin superstructure, of which these section A sequences are a constituent part. In replication-defective λ *ti*12 (ref. 3) a transversion at position 1,100 (ref. 6) points out the only single nucleotide so far known to be of functional importance. Quite consistently, in constructing the two different DNA cloverleaves, the constant ACA group surrounding this position had to be placed into an identical situation with respect to both structures, as in fact both of the cloverleaves are located almost exactly in register with respect to the central sections, or to the section C DNA hairpins (36 compared with 35 base pairs for the smaller, 39 compared with 37 base pairs distance for the larger section C hairpins).

It is certainly obvious that our model for the activated, single-stranded functional structure of the lambdoid origin of replication as deduced from DNA sequences and from genetic studies[9], needs to be studied further biochemically, both *in vivo* and *in vitro*. However, sandwiching of a primase recognition site which is kept in a single-stranded, rigid conformation by two different initiator proteins, which in turn are complexed to two adjacent, elaborate DNA binding sites, seems to be able to explain both the basic structural as well as functional designs of such replicator signal elements.

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Domains and the hinge region of an immunoglobulin heavy chain are encoded in separate DNA segments

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A 6.8-kilobase DNA fragment containing the sequence coding for the constant region of the mouse immunoglobulin γ_1 heavy chain was cloned from total cellular DNA. Electron microscopic and nucleotide sequencing studies showed that the three protein domains and the hinge region are encoded in separate DNA segments.

SOME higher cell genes consist of informational DNA interspersed with silent sequences. Eukaryotic cistrons are often transciption units containing alternate regions to be excised from the primary transcript, introns, and to be left in the mature messenger RNA for expression, exons[1]. The first indication of such a mosaic gene structure was obtained in the 28S ribosomal RNA gene of *Drosophila melanogaster*[2,3]. This observation was followed by discoveries of an intron within the RNA leader regions of the human adenovirus late genes[4,5]. Introns were also found in the protein-encoding regions of mouse immunoglobulin λ-chain genes[1,6], rabbit and mouse β-globin genes[7,8], hen ovalbumin genes[9,10], and in yeast tRNA genes[11,12]. More examples are accumulating. These studies show that introns are widespread in higher cells and their viruses, but the functions and origin of introns remain unclear.

Both light and heavy chains of immunoglobulin molecules consist of two functionally differentiated regions: the amino terminal variable (V) region and the carboxyl terminal constant (C) region. The V region recognises and binds antigens while the C region exerts various effector functions such as interaction with cell surface membrane and complement. Amino acid sequence studies have shown that both light and heavy chains consist of homology units[13,14]. For instance, the C region of a γ-class heavy chain contains three homology regions, CH1, CH2 and CH3, each of which is similar in size (about 110 residues) and homologous to the C region of a light chain[15]. Furthermore, the V regions of both light and heavy chains may also be related to the C region based on the arrangement of intrachain disulphide bonds and their size, and the presence of a

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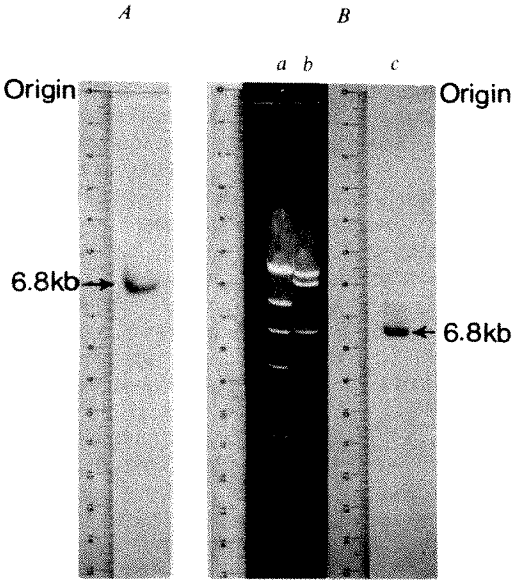

Fig. 1 Southern hybridisation of pH21-1 cDNA to myeloma MOPC 21 or cloned IgCγ_1-1 DNA. *A*, 2 mg of high molecular weight DNA extracted from myeloma MOPC 21 was digested to completion with *Eco*R1 and separated on 0.8% horizontal agarose gel slab (20×40×1 cm) in TA buffer (40 mM Tris-HCl, 5 mM Na-acetate, 1 mM EDTA, *p*H 7.9) at a constant current of 50 mA for 4 days. To detect the γ_1 gene-positive DNA fragments, a 0.5-cm wide section of the gel was cut out from the centre in parallel with the direction of electrophoresis, and was blotted onto a nitrocellulose filter (Schleicher-Schuell, BA85) for 3 h as described by Southern[20]. The filter was incubated at 65 °C for 12 h in the presence of 2×10^7 c.p.m. of denatured, nick-translated pH21-1 cDNA (specific activity 2×10^8 c.p.m. per μg) as described elsewhere[19]. The figure shows an autoradiogram of the filter after 6 h of exposure. The molecular size of the γ_1 gene-positive band was estimated from its relative electrophoretic mobility to *Hin*dIII fragments of phage λ DNA. For the cloning, DNA was recovered from the preparative slab gel as follows. Hydroxyapatite (Bio Rad, DNA grade) washed with TA buffer was mixed with an equal volume of 0.8% melted agarose (in TA buffer) and the mixture was poured into the 0.5-cm wide vertical slot in the centre of the gel slab and into a parallel slot of 0.5-cm width located near one edge of the gel. The gel slab was rotated 90° and electrophoresis was continued at 150 mA for 4 days to let DNA fragments migrate into and bind to the embedded hydroxyapatite. Hydroxyapatite containing γ_1 gene-positive DNA fragments was identified from the autoradiogram of the Southern gel blot, and a 0.5-cm thick slice of the hydroxyapatite cake in this region was cut out. The slice was suspended in 1 ml of saturated KI solution and heated at 65°C for 15 min to melt the agarose. The hydroxyapatite was collected by centrifugation and was washed with 3 ml of 10 mM KPO_4 buffer (*p*H 6.9). The DNA was eluted from the hydroxyapatite with 0.5 ml of 0.6 M KPO_4 (*p*H 6.9) buffer and the hydroxyapatite was removed by centrifugation. The DNA was dialysed against 10 mM Tris-HCl *p*H 7.9 containing 1 mM EDTA and was concentrated by ethanol precipitation. *B*, 0.5 μg of cloned Ig Cγ_1-1 DNA was digested with *Eco*RI, separated in a 1% agarose gel and stained with 0.5% ethidium bromide (lane *b*). DNAs were transferred onto nitrocellulose filter and hybridised with 2×10^6 c.p.m. of nick-translated pH 21-1 cDNA (2×10^8 c.p.m. per μg) (lane *c*). *Hin*dIII-digested phage λ DNA (0.5 μg) was separated in the same gel as a size marker (lane *a*). The third largest *Hin*dIII-λ fragment is 6.6 kilobases long.

weak homology between these regions[13,15]. These studies suggested two important points on the structure and evolution of immunoglobulin molecules. First, homology regions may have similar three-dimensional structures, each consisting of a compact globular domain. This hypothesis was directly confirmed by X-ray crystallography[16,17]. Second, the presence of the internal homology suggested that both light and heavy chain genes evolved by duplication of a primordial gene that codes for a polypeptide chain with a size of a single homology unit[13]. Our previous studies on λ and κ light chain genes demonstrated that the V and C domains are encoded in separate exons[6,18,19]. We here report that the three C region domains of mouse γ_1 heavy chain as well as the chain-linking hinge region between the CH1 and CH2 domains are encoded in separate DNA segments.

Identification and cloning of an *Eco*R1 fragment carrying a Cγ_1 gene

High molecular weight total cellular DNA extracted from MOPC 21 myeloma was digested to completion with *Eco*R1 and the resulting DNA fragments were analysed by the Southern gel blotting technique[20] using as the hybridisation probe a nick-translated cDNA clone (pH21-1) prepared from MOPC 21γ_1 chain mRNA. As shown in Fig. 1*A*, we detected a single band of 6.8 kilobases that hybridised with the cDNA probe. Rogers *et al.* sequenced the γ_1 cDNA and found that it contains sequences coding for part of the Cγ_1 region that extends from amino acid residue 287 to 439[21]. We conclude that the 6.8-kilobase *Eco*R1 fragment contains at least part of the Cγ_1 DNA sequence.

DNA fragments in the 6.8-kilobase fraction were recovered from the preparative agarose gel and were ligated with the isolated DNA arms of an EK-2 vector, phage λgt WES[22]. The recombinant DNAs were packaged *in vitro* into phage coats[23] and plaques were screened by *in situ* hybridisation using the nick-translated Cγ_1 cDNA clone as probe[24]. Screening of about 2×10^5 independently arising plaques lead to the identification of eight positive clones. When digested with *Eco*R1, DNA from the eight phage clones gave, in addition to the left and the right DNA arms of the vector, a 6.8-kilobase DNA fragment that hybridised with the Cγ_1 cDNA probe. The results of one of the eight phage clones, IgCγ_1-1, are shown in Fig. 1*B*.

Table 1 Length measurements of R-loop molecules

Segment	Length (kilobases)	*N*
A	3.29±0.13	48
B	1.92±0.12	68
R1	0.28±0.05	64
R2	0.34±0.05	53
R3	0.42±0.08	60
I1	0.51±0.09	45
I2	0.18±0.03	14
I1a	0.37±0.06	29

The R-loops R1–R3 were measured in double-stranded molecules, in which the intervening sequence I_1 was stretched out or seen as an intron-loop, and I_2 was seen only in stretched out molecules (Fig. 2*b*). In the single-strended DNA–mRNA hybrids the intron I_{1a} is shortened because of the annealing of the hinge coding region. I_{1b} is not measurable, but probably is about 100 base pairs long. The hinge region must be within 140 base pairs from the 5′ end of the R2 coding region. Values are given ±s.d. *N*, numbers of measured molecules. Measurements of R-loop molecules were made with a Numonics digitiser and the data analysed with a Hewlett Packard HP9825 calculator.

The Cγ_1 gene is split

The position of the Cγ_1-coding region in the 6.8-kilobase DNA fragment of the IgCγ_1-1 clone was determined by electron microscopy. R-loop molecules were formed between the cloned DNA and a γ_1 chain mRNA purified from MOPC 21 myeloma cells[25]. Figure 2 shows the various R-loop structures observed. Most of the molecules have three small R-loops, R1, R2 and R3, that are separated by two double-stranded intervening DNA segments (I1 and I2) (Fig. 2*a*). In all molecules a short RNA tail could be observed at the largest R-loop (R3), and in some molecules a longer RNA tail extends also from the shorter R-loop (R1).

The sites of R-loops and intervening DNA segments are summarised in Table 1. The intervening DNA segment between R2 and R3 (I2) is very short and cannot be visualised as a clear double-stranded loop, rather it appears as a little knob separating the two R-loops. The length of the two intervening DNA segments can best be measured in those hybrids where several mRNA fragments have annealed to the same DNA fragment (Fig. 2*b*, *c*). The lengths of R1 (280 base pairs) and R2 (340 base pairs) roughly correspond to the size of a DNA segment coding for a single domain (100–120 residues) of an immunoglobulin chain, while the length of R3 (420 base pairs) is slightly larger. We interpret the various parts of the observed R-loop structure in the following way. R1 and R2 correspond to the DNA segments coding for the CH1 and CH2 domains, respectively, while R3 probably encodes the CH3 domain and the presumed 3′-untranslated region of γ_1 chain mRNA. This interpretation is supported by the length and the location of the two RNA tails observed in the intact R-loop structure (Fig. 2*a*). The short (~200 bases) and the long (~400 bases) RNA tails can be interpreted to represent the poly A and the V-coding sequence at the 3′- and the 5′-end of the mRNA.

Correlation between exons and the domain- and hinge-coding DNA segments

In order to confirm the interpretation of the R-loop structure described above, we constructed a restriction enzyme cleavage map of the IgCγ_1-1 clone and determined the nucleotide sequences of the relevant regions (Fig. 3*b*, *c*).

In determining the nucleotide sequences we first concentrated on the five regions (indicated in Fig. 3*c* as I, II, IV, V, and VI) that cover the ends of the three major exons. In so doing we discovered that coding at the right end (see Fig. 3*c* for orientation) of exon 1 and the left end of exon 2 leaves about 13 amino acid residues around the hinge region (Fig. 3*d*) unaccounted for. DNA sequencing revealed an additional exon (exon H) that codes for this cysteine-rich region within the 0.5-kilobase DNA segment separating R1 from R2. Electron micrographs of single-stranded DNA-mRNA hybrids (Fig. 2*d*, *e*) are consistent with the presence of exon H. When γ_1 mRNA was hybridised to denatured IgCγ_1-1 DNA, it annealed to the hinge region, thus forming another short intron I1b that is similar in size and appearance to I2. We thus call the four protein-encoding DNA segments exon 1, H, 2 and 3, and the three non-coding intervening DNA segments intron B, C and D as indicated in Fig. 3*c*. We also call the non-coding DNA segment to the left of exon 1 intron A because we anticipate the presence of another short exon further upstream (that is, towards the left in Fig. 3) that codes for the V–C junction region (J region)[18] in analogy to the structure of a λ light chain gene.

The DNA sequences in the six regions (I–VI) indicated in Fig. 3*c* are shown in Fig. 4. Also shown in Fig. 4 are part of the amino acid sequences of MOPC 21γ_1 chain as determined by Adetugbo[26], and the nucleotide sequences predicted from the amino acid sequence. Coding in exon 1 begins with Ala at residue 121 and ends in Val at residue 215. Coding in exon H starts with the Val and ends in Val at residue 228. Coding in exon 2 starts with the Val codon and ends in Gly at residue 335. Finally, coding in exon 3 starts with the Gly residue. The entire

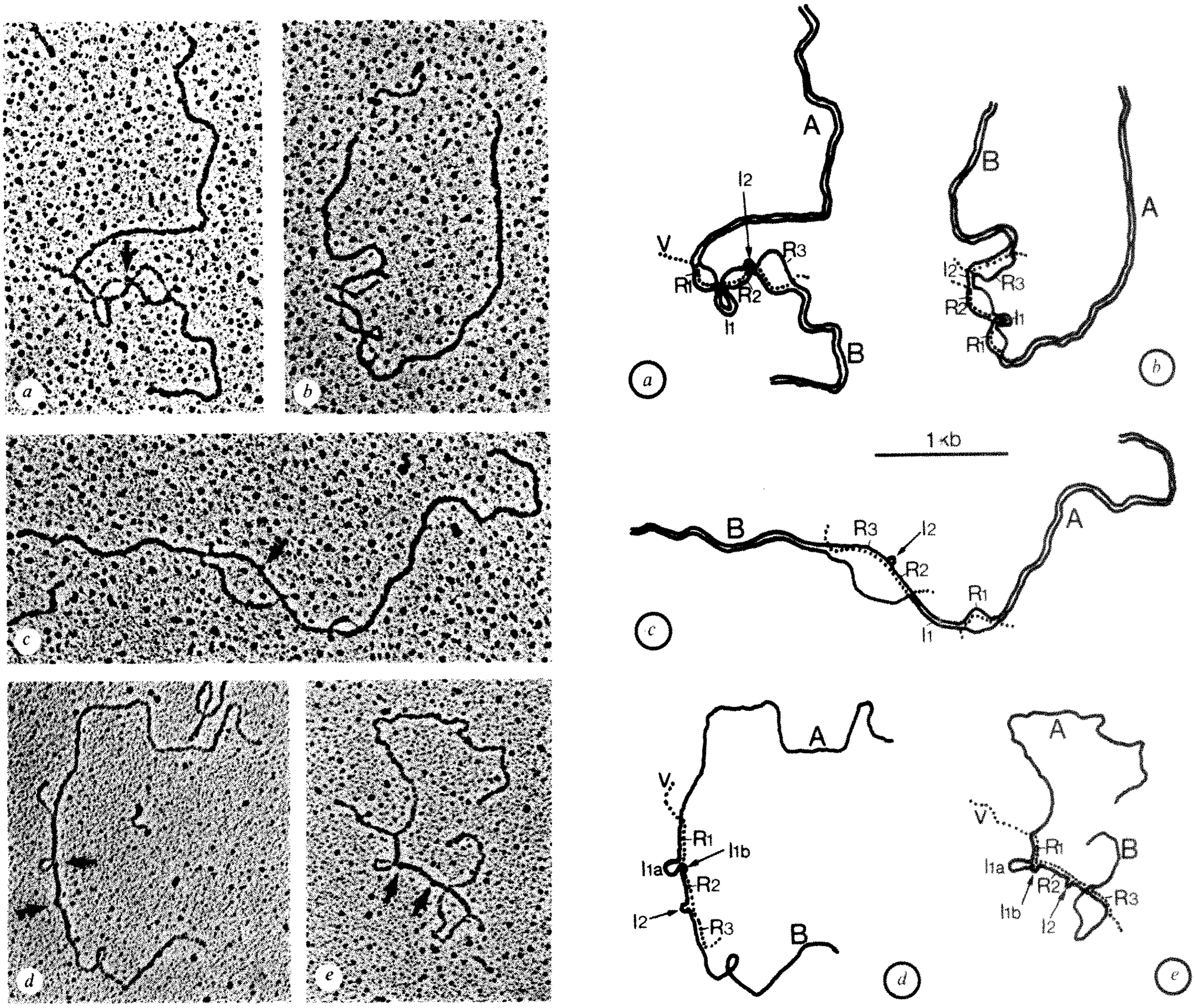

Fig. 2 Left: electron micrographs of R-loop molecules (*a*–*c*) and RNA–DNA hybrids (*d*, *e*). The *Eco*RI-digested IgCγ_1-1 DNA was incubated with MOPC21 γ_1 mRNA under R-loop conditions at 57 °C[18]. Single-stranded DNA–mRNA hybrids were formed after heat denaturation and incubation of the R-loop mixture at 60 °C. The molecules were mounted for electron microscopy by the formamide-cytochrome *c* spreading method[40,41]. Three R-loops R_1, R_2, R_3 are separated by two intervening sequences I_1 and I_2. When two separate mRNA molecules hybridise to different R-loops, the intervening sequences are stretched out and can be measured (*b*, *c*). In single-stranded hybrids (*d*, *e*) it is possible to resolve also the small intron I_{1b} which separates the hinge coding region from the intron I_{1a}, and which appears as a small knob beside I_{1a}. Right: schematic interpretation of the hybrid molecules.

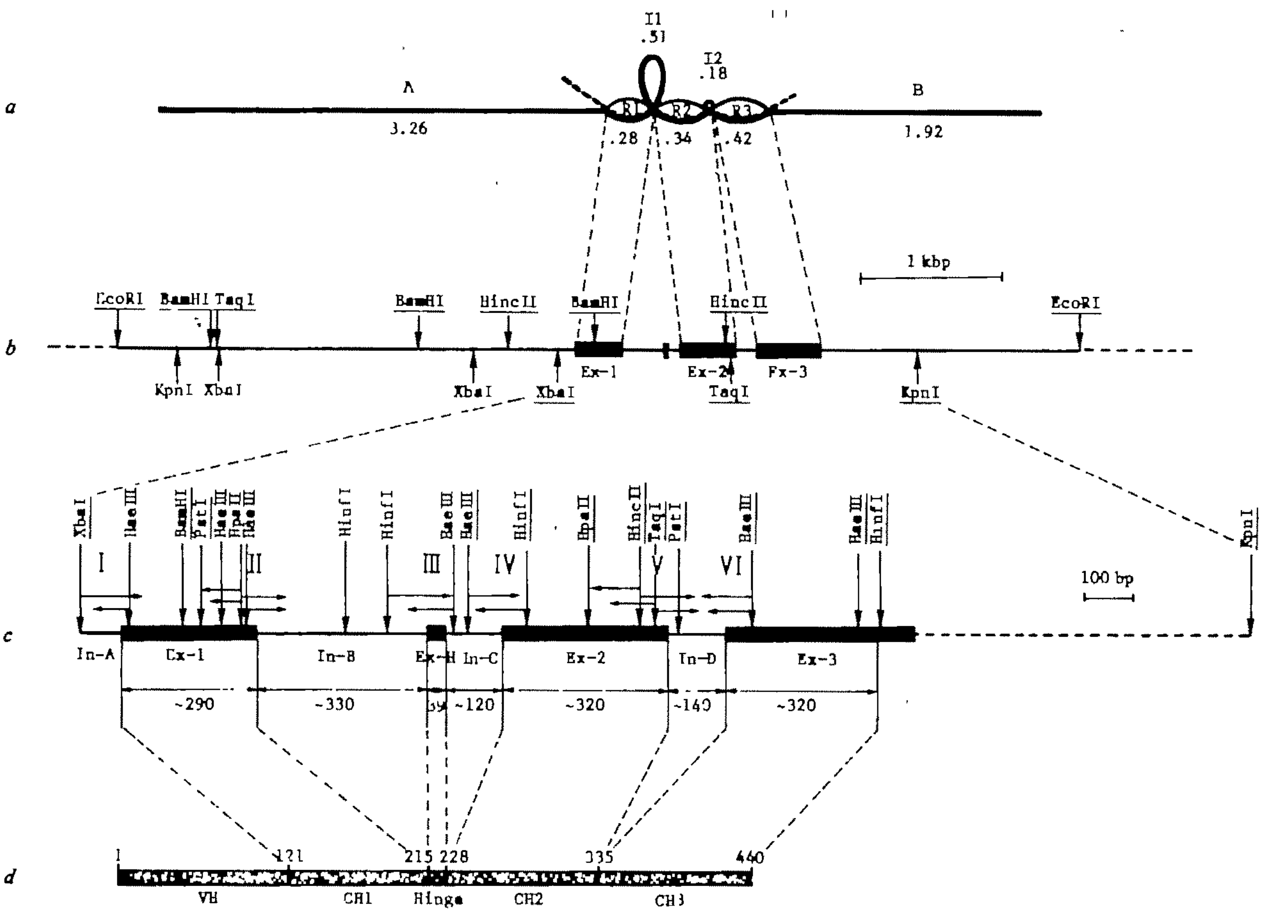

Fig. 3 *a*, R-loop map of the 6.8-kilobase insert. Heavy broken lines and heavy solid lines represent MOPC 21 γ_1 mRNA and double-stranded DNA, respectively. Duplexes between fine solid lines and broken lines are DNA–RNA hybrids. Fine solid lines are replaced single-stranded DNA The numbers indicate lengths of various parts in kilobases. *b*, A map of the 6.8-kilobase insert obtained by the combination of restriction enzyme digestion and R-loop formation. The whole IgCγ_1-phage DNA was digested either with one of the indicated enzymes or with that enzyme plus *Eco*RI and separated in a 1% agarose gel. Comparison of the single and the double digestion patterns allowed us to localise the outmost cleavage sites of each enzyme on the 6 8-kilobase insert Additional cleavage sites were identified by comparison of the electrophoresis patterns obtained after digestion of the isolated 6.8-kilobase insert with one enzyme or various combinations of two enzymes. To correlate the restriction map with the R-loop map, we analysed R-loops formed between the γ_1 mRNA and the insert which had been digested with respective enzymes. The cleavage sites identified this way are underlined. R1, R2 and R3 in (*a*) correspond to Ex1, Ex2 and Ex3 in (*b*) respectively. *c*, Detailed restriction enzyme map around the exons Sites for *Hae*III and *Hin*fI were mapped in the five isolated pieces generated by *Taq* and *Bam* cleavage of the 6 8-kilobase insert. For each piece in turn, parallel digests were done with *Hae*III and *Hinf*, and the products were electrophoresed on 6% polyacrylamide. They were compared with similar digests of the whole insert. By noting the size of the fragments which were present only in the digests of the whole insert, and of the fragments generated from them in the *Bam*- and *Taq*-cut material, most of the sites around the *Bam* and *Taq* sites could be located. Within and between exon 1 and exon 2 all the remaining sites could also be identified, since there was only one way in which the fragments observed on single and double digests could be self-consistently ordered Two small (30–40 base pairs) *Hae*III fragments had to be postulated to make up the total length of this region One of these was confirmed, and some remaining ambiguities were resolved by labelling the 5′ ends of *Bam*-cut pieces of the 6.8-kilobase insert with [γ-^{32}P]ATP, cutting with *Hinf*, isolating the single end-labelled fragments by preparative polyacrylamide gel electrophoresis, and analysing polyacrylamide gel electrophoresis, and analysing partial digests of them by *Hae*III. Within exon 3, the 217-base pair *Hae*III fragment was identified by comparison with a *Hae*III digest of pH21-1, the cDNA clone, in which the same fragment is present The underlined cleavage sites were confirmed by Southern gel blotting analysis using the pH21-1 cDNA probe. The lengths (in base pairs) of introns and exons are based on the DNA sequencing data as well as on the size estimation of restriction fragments. Hinge coding region (exon H) was located between exon 1 and exon 2 by DNA sequencing (see text). The roman letters indicate six regions that were sequenced. Horizontal arrows indicate actual fragments used for sequencing. In, intron, Ex, exon. *d*, Correlation of domains and hinge of MOPC 21γ_1 heavy chain to four exons of Cγ_1-1 DNA. Numbers represent the amino acid positions at the domain-hinge or domain–domain boundaries Numbering starts with the first NH$_2$-terminal asparagine of the γ_1 chain

amino acid sequence of the MOPC 21 γ_1 chain was determined by Adetugbo[28]. While the presence of the sequence homology among the three C region domains is obvious, the exact domain boundaries are ambiguous. Nevertheless, examination of the internal sequence homology in the γ_1 chain and comparison of its sequence with that of a human γ_1 chain[15], for which the three dimensional structure of the Fab fragment is available[16,17] makes it possible to draw every domain-boundary within short regions that contain no more than a few amino acid residues. Thus CH1–hinge, hinge–CH2 and CH2–CH3 boundaries can be placed around residues 214–218, residues 227–228, and residues 332–335, respectively. Furthermore, the amino terminal end of the CH1 domain is around residues 115–122. The nucleotide sequences show that in all cases coding ends or begins with a residue that lies within one of these inter-domain regions deduced from structural analysis of the protein. Since it is extremely unlikely that such a correlation is a coincidence, we conclude that each of the three Cγ_1 domains and the hinge region are encoded by separate exons. Indeed one may look at it in the opposite sense and say that the exact ends of domains and the hinge region are defined by the intron–exon boundaries on the DNA.

In the coding regions the fit between the determined and the predicted nucleotide sequences is good. However, note the discrepancies in 10 codons in the regions shown in Fig. 4. Of the 10 differences, 9 are due to exchange or permutation of residues at nearby positions (for instance the Pro–Ser exchange in exon 1) and the tenth is between Ser and Thr. Thus these discrepancies are probably due to errors in determination of the amino acid sequence. Arg–Lys exchange in exon 3 was found previously[21] in the sequence of cDNA clone, pH21-1.

Are there any additional introns within the DNA segments coding for the three domains? The entire sequences of exon 1 and exon 2 segments have been determined (K. H. and H. S., unpublished), and no coding gaps were found in these regions. In exon 3, sequencing has not been completed, but the 220-base pair *Hae*III–*Hae*III fragment arising from exon 3 co-migrates with the equivalent fragment from the pH21-1 cDNA clone in a 6% acrylamide gel. It seems that there is no intron within domain-encoding segments.

Nucleotide sequences at the splice sites

The coding gaps in split genes are considered to be eliminated during processing of the primary RNA transcript by splicing of exon sequences[27–29]. The nucleotide sequences of the putative splice sites in the Cγ_1 gene are listed in Fig. 5 together with the splice site sequences of the λ_I and λ_{II} light chain genes which we previously determined[1,30]. As in all other known split genes, the exact points of coding interruption are ambiguous because of the redundancy near the opposite ends of the introns. Thus a single nucleotide G is repeated near the two ends of intron B, a dinucleotide AG near the ends of introns C, and a trinucleotide AGG near the two ends of intron D. By comparing sequences near the ends of six ovalbumin gene introns Breathnach *et al.*[31] found that it is always possible to choose cutting points so that the base sequence of an intron begins with GT and ends with AG. This rule applies to the two small introns of λ chain genes[1,30], the six introns of SV40[32] and the small introns of β-globin genes[33]. While intron C and intron D of the Cγ_1 chain gene also obey this rule, intron B does not. Because of the G repetition intron B either begins with GA and ends with CA or begins with AG and ends with AG. Another generalisation that can be made for the sequence near the ends of introns is an apparent presence of a basic sequence of several bases from which all known marginal sequences can be derived with no more than one or two base changes[31,34]. In particular we noted that all three introns of immunoglobulin λ chain genes have the sequence AGGTNAG near the 5′ end (relative to the direction of transcription)[30]. While introns C and D of the Cγ_1 gene have the same sequence at the analogous positions, intron B seems to have a quite different sequence. Sequence similarity near the 3′-end of introns is somewhat less, but YAG is shared by all introns of the λ and the γ_1 chain genes. In addition, as in most other introns, sequences rich in pyrimidines precede the basic sequence near the 3′ ends of Cγ_1 introns.

Deletion mutants

The correlation between exons and domain- or hinge-encoding DNA segments immediately drew our attention to the fact that mutant or variant immunoglobulin chains are often found in

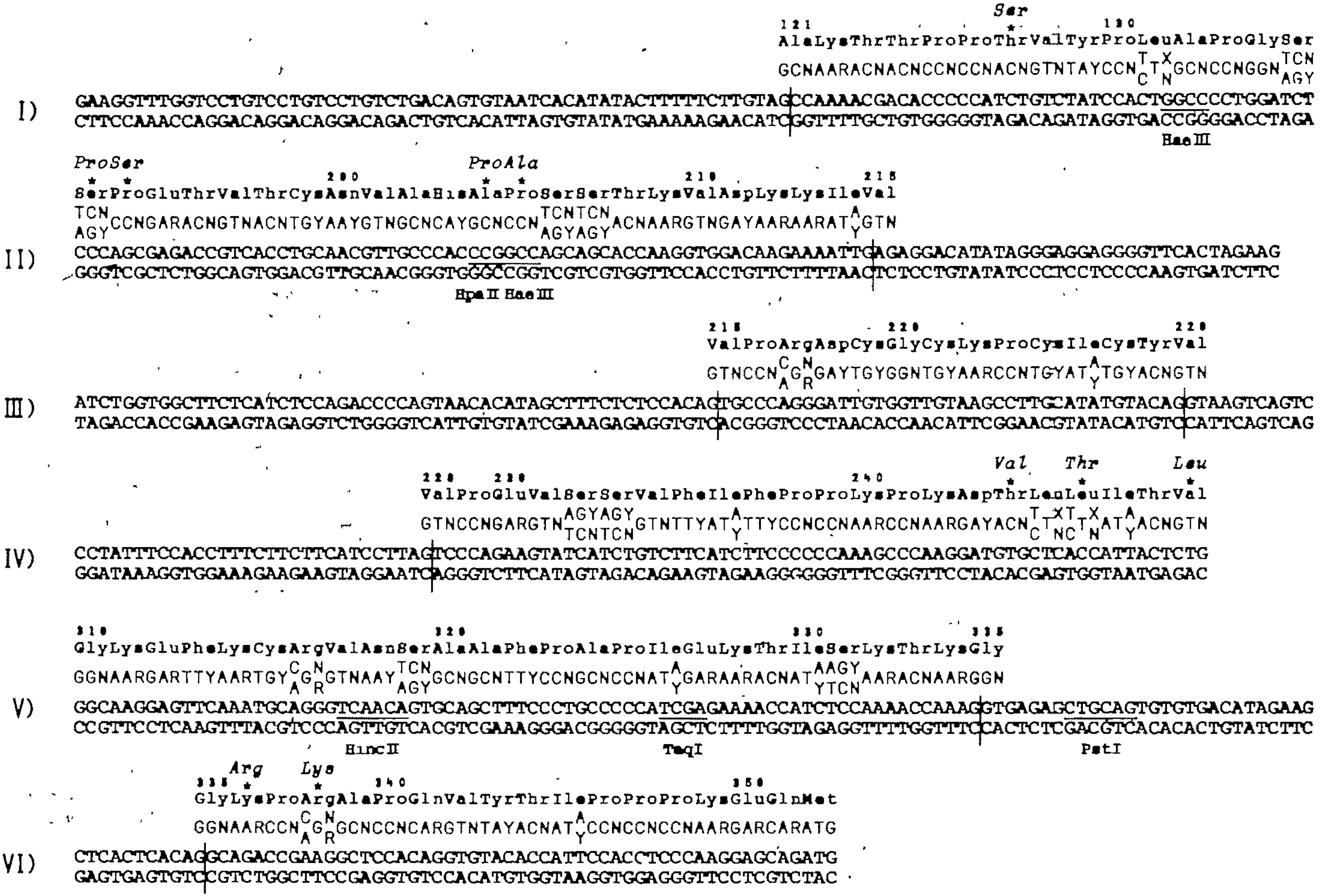

Fig. 4 Partial nucleotide sequences of clone IgCγ_1-1 Nucleotide sequences of the six regions, I, II, III, IV, V, and VI indicated in Fig. 3*c* are shown. For DNA sequencing, 2 mg of phage IgCγ_1-1 DNA was cleaved with *Kpn*I and *Xba*I and separated on a 5% polyacrylamide gel (20×40×0.5 cm). The 2.5-kilobase *Xba*I–*Kpn*I fragment (see Fig. 3*c*) containing the Cγ_1-coding sequences was eluted electrophoretically from the gel and washed by ethanol precipitation. The fragment was then cleaved with *Hin*fI, *Hae*III, *Hpa*II, *Taq*I or *Hin*cII, treated with bacterial alkaline phosphatase (Worthington), end-labelled with T_4-polynucleotide kinase (P–L Biochemicals) and [γ-^{32}P]ATP (Amersham), and sequenced by the method of Maxam and Gilbert[42] with a few modifications. Cleavage reaction at A and G was performed as follows (A. Maxam, personal communication). A mixture of end-labelled DNA and 1 μg of carrier calf thymus DNA was incubated in 23 µl of 87 mM pyridine formate *p*H 2 at 20 °C for 90 min. After lyophilisation, 20 µl of 0.5 M piperidine was added and the mixture was heated at 90 °C for 30 min in a sealed capillary. The sample was then lyophilised three times, dissolved in 10 µl of 0.1 M NaOH and 1 mM EDTA, and combined with 10 µl of urea–dye mixture. For G, C and T and C reactions, the conventional method was used except that Mg-acetate was omitted from dimethyl sulphate and hydrazine stop solutions. Reacted samples were separated in thin polyacrylamide gels (30×40×0.035 cm) at 1.5 kV as described by Sanger and Coulson[43] The figure also shows partial amino acid sequences of the γ_1 chain from myeloma MOPC 21[26] as well as the nucleotide sequences predicted from the amino acid sequence. Amino acids which do not match those predicted from our nucleotide sequence are indicated by asterisks, and the predicted amino acids are presented in italics. Vertical lines indicate possible intron–exon boundaries. Cleavage sites for some restriction endonucleases are underlined. Amino acid numbering begins with the asparagine at the NH_2-terminal end of the mature γ_1 chain from MOPC 21. N: A, G, C, or T. R: A or G. Y: C or T.

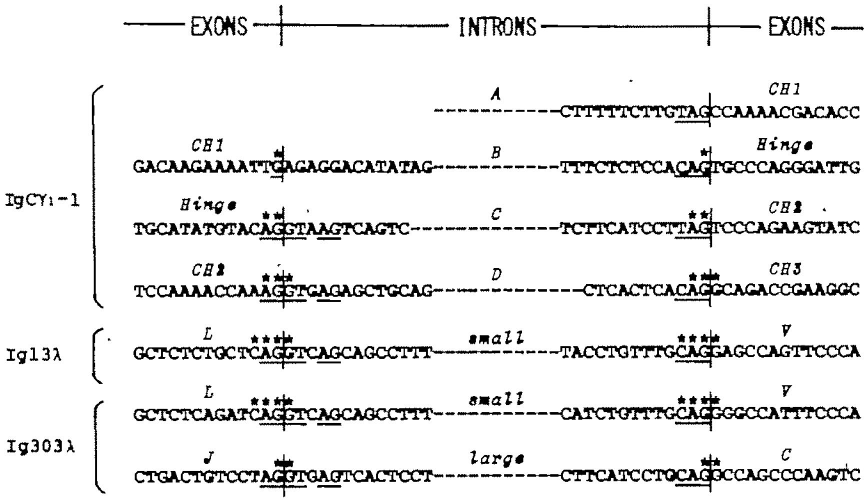
EXONS
INTRONS
EXONS
IgCγ1-1
A
CH1
------------CTTTTTCTTGTAGCCAAAACGACACC
CH1
B
Hinge
GACAAGAAAATTGAGAGGACATATAG------------TTTCTCTCCACAGTGCCCAGGGATTG
Hinge
C
CH2
TGCATATGTACAGGTAAGTCAGTC------------TCTTCATCCTTAGTCCCAGAAGTATC
CH2
D
CH3
TCCAAAACCAAAGGTGAGAGCTGCAG------------CTCACTCACAGGCAGACCGAAGGC
Ig13λ
L
small
V
GCTCTCTGCTCAGGTCAGCAGCCTTT------------TACCTGTTTGCAGGAGCCAGTTCCCA
Ig303λ
L
small
V
GCTCTCAGATCAGGTCAGCAGCCTTT------------CATCTGTTTGCAGGGGCCATTTCCCA
J
large
C
CTGACTGTCCTAGGTGAGTCACTCCT------------CTTCATCCTGCAGGCCAGCCCAAGTC

Fig. 5 Sequences around the splice sites of three immunoglobulin genes. Nucleotide sequences around the splice sites of the IgCγ_1-1 clone as well as those of Ig13λ[1] (contains an embryonic $V_{\lambda II}$ gene) and Ig303λ[30] (contains a myeloma (V+C) λ_I gene) are summarised. Intron–exon boundaries defined by the GT–AG rule[31] are indicated by vertical lines. The short 'common' sequences near the 5'- or 3'-end of introns are underlined. In case of intron B which does not follow the GT–AG rule, the boundaries indicated are based on the assumption that the intron ends with AG (see text). Nucleotides repeated near the opposite ends of an intron are marked with asterisks. CH1, CH2 and CH3 designate the three constant region domains of the γ_1 heavy chain. L, V, J, and C designate the hydrophobic leader, variable, joint, and constant region of the λ_I or λ_{II} light chain

which an entire domain or the hinge region is deleted. Franklin and Frangioni reported several examples of so-called human heavy chain disease proteins with such characteristics[35]. In the present context, one mouse γ_1 chain mutant isolated from cultured MOPC 21 myeloma cells is of particular interest[36]. This mutant, called IF2, lacks the CH1 domain extending from residue 121 to residue 214. The deleted amino acids are exactly those that are encoded by exon 1 (Fig. 4). The so-called C_κ fragment found in myeloma MPC 11 may be another example[37]. The sequence of the amino terminal region of the C_κ fragment that had been synthesised in a cell-free translation system under the direction of purified C_κ fragment mRNA has been determined [38,39]. The sequence suggests that the C_κ fragment is synthesised in the cells as a precursor carrying a hydrophobic leader peptide at the amino terminus. In the precursor a 17-residue long leader is directly attached to the C_κ region peptide. Our previous results showed that both the leader and the V regions are encoded in separate exons in mouse λ chain genes[1,30]. If the same applies to κ chain genes, the C_κ fragment precursor may be considered as a deletion variant in which the entire peptide encoded in the V-coding exon is deleted.

Assuming that the splicing enzyme can potentially bridge two non-adjacent exons, then all deletions which begin in one intron and end in another could generate a single mutant protein in which the entire polypeptides encoded in exons between the two introns are deleted. Alternatively, a mutation at or near a splice site, whether it is a deletion or a base change, could perturb the splicing pattern such that the entire sequence in one exon is skipped in the mRNA. For instance, certain mutations near the intron B–exon H boundary may block splicing of exon 1 with exon H and in turn may promote its splicing with exon 2. Cloning and analysis of the relevant DNA fragments from mutant cells will test these models directly.

Evolution of immunoglobulin genes and origin of introns

How splicing arose in evolution is unknown. Crick has suggested that splicing was originally a protection mechanism, evolved by the host organism to reduce the damage being inflicted by the insertion of nonsense sequences in the middle of the gene (F. Crick, personal communication). In this hypothesis, the introns are viewed as DNA elements that were once inserted more or less randomly within a pre-existing gene. The placement of introns in ovalbumin and β-globin genes indeed seems to be random. However, the presence of introns at the regularly spaced boundaries of domain-coding DNA segments and at the boundaries of the domain- and hinge-encoding DNA segment is in apparent contrast to such a 'random' insertion of DNA elements. To explain the regularity observed in the location of the introns we propose the following scheme as a possible and likely process of the evolution of an immunoglobulin heavy chain gene. It is likely that when the putative primordial gene duplicated, the unit of duplication was substantially larger than the unit of translation. As DNA duplication is likely to involve some form of illegitimate recombination which makes use of accidental short sequence homology, there is no *a priori* reason why a domain coding DNA segment would be duplicated immediately adjacent to itself. Most probably, the duplicated DNA would contain a spacer between the two domain-encoding DNA segments. One obvious way to fuse the DNA segments is to delete the spacer. But, for exactly the same reason as given above for the duplication step, such a deletion is probably a rare event. Instead, the organism might very well have resorted to a pre-existing splicing mechanism that might have developed under an evolutionary pressure such as that suggested by Crick.

How did the necessary splice sites arise at or near the ends of the domain-encoding DNA segments? One possibility is the primordial domain gene was itself an intact exon bound by splice sites. Alternatively, splice sites might have been created *de novo* by drift at the proper regions after duplication. In most cases, there seems to be a basic sequence of four to six bases to which sequences near the margins of introns are related in varying degrees. While it may not be difficult to imagine that a proper sequence of this length is generated rather frequently by drift, the specificity provided by these sequences cannot be sufficient. The same sequence is also found within exons[34]. The obvious possibility is that combination of some higher order structure and the sequence information determine the specificity.

To generate the four-domain heavy chain gene the process outlined above is imagined to have been repeated. An additional aspect of the evolution of heavy chain genes is the generation of the hinge-coding exon. While it is possible to imagine that this exon arose from part of the ancestral intron between exon 1 and exon 2, there are two more interesting possibilities. One is that the hinge evolved by reduction of an extra C region domain corresponding to the CH2 domain which is found in place of hinge in μ and ε chains[44]. The other is the DNA segment containing the hinge-coding sequence with or without ready-made splice sites at its margins was inserted into the ancestral intron between the exon 1 and exon 2.

Whichever variation of the model described above is more likely to be correct, it suggests that the splicing mechanism, with or without recombination at the DNA level, enables an organism to create a new gene by assembling DNA pieces each coding for a polypeptide chain of some functional use[1]. If such a gene creation process indeed took place in evolution, some of the present day introns should be regarded as spacers between assembled exons and not as insertion sequences *per se*. As suggested by the hinge-coding exon and the hydrophobic leader-coding exon[1,30], the correlation between a clearly defined protein domain and an exon is not a necessary element of the proposed gene creation process. A DNA segment that codes for a protein of some function and flanked by splice sites, whether they arose from the margins of insertion elements or by mutational drift, can combine with another such segment coding for a protein of a similar or an entirely different function. The function could be for binding with a specific ligand, for providing disulphide bridges or even for simply providing a peptide that can stabilise the overall structure of the newly created protein.

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letters

Photon decay in curved space-time

RECENT astrophysical observations[1,2] have raised doubts as to whether the large redshifts of distant galaxies (the Hubble redshift) are due entirely to cosmological expansion. The strongest argument[3] in favour of cosmological expansion is that there is no known hypothesis consistent with the laws of physics (other than the Doppler shift hypothesis) that can explain the observed redshifts. An alternative explanation—a gradual energy loss of photons due to their interaction with curved space-time—is considered here. The basic premise is that because photons have a finite spread they are subject to tidal stresses and that this provides a mechanism for the transfer of momentum from the photon to the mass producing curved space-time. Any transfer of momentum without an equivalent transfer of energy will destroy the concept of the photon as a single elementary particle. It is therefore postulated that the interaction of the photon with curved space-time causes it to lose energy in the form of very low energy secondary photons. As well as providing an explanation for the Hubble redshift this hypothesis can also explain the solar limb effect, that is, the increasing redshift of solar spectral lines as the viewpoint approaches the limb of the Sun.

Most texts that consider the passage of light in curved space-time equate the trajectory of a photon to that of a light ray, thereby neglecting any lateral spread the photon may have. If we exclude the possibility of infinite energy density then in some sense not yet defined a photon must have a finite non-zero cross-sectional area. A better model for the photon is a bundle of light rays. The effect of the tidal stresses on this bundle is shown by their relative deviations from a central reference geodesic. An appropriate coordinate system is an orthonormal Riemann coordinate system that is parallel transported along this central geodesic. Furthermore, every point along the central geodesic is uniquely labelled by the value of an affine parameter τ. As the deviations that are linear in τ given only reversible focusing (or defocusing) effects they will be ignored and we shall consider only the irreversible deviations that are quadratic in τ. These are given by the familiar equation for geodesic deviation.

The propagation of light in curved space-time is identical to propagation in a medium with a heterogeneous refractive index[4]. As the solution of Maxwell's equations is much easier for the latter case this approach is adopted here. The refractive index that gives the same geometric behaviour of light rays as the deviation of geodesics is radially symmetric about the axis of propagation and has the form

$$n = (1 - \varepsilon^2((x^2)^2 + (x^3)^2))^{1/2} \tag{1}$$

where ε is a small constant and the central geodesic is along the x^1 axis. Then to order ε propagating wave solutions can be found with a gaussian amplitude distribution in the radial direction and requiring the dispersion relationship

$$\omega_0^2 = c^2 k_0^2 + 2\varepsilon\omega_0 c \tag{2}$$

where ω_0 is the circular frequency and k_0 is the wavenumber. This gives a group velocity of $v = (1 - \varepsilon/k_0)c$, where c is the velocity of light along the reference geodesic.

Equation (2) can be interpreted as giving the wave packet an effective mass of $m = (2\varepsilon/k_0)^{1/2}(E_0/c^2)$ where $E_0 = \hbar\omega_0$ is the energy of the wave packet. The basic hypothesis here is that the same result applies to a photon in curved space-time and that this effective rest mass is just the energy of two secondary photons that are being emitted. Two secondaries are required to preserve spin. Each secondary has a wavenumber $k = (\frac{1}{2}\varepsilon k_0)^{1/2}$.

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The Heisenberg uncertainty principle ($\Delta k_0 \Delta x \simeq 1$) can be applied to one of the secondary photons to estimate the rate of production of these secondaries and hence the rate of energy loss by the primary photon. Then with $\Delta k_0 = k = (\frac{1}{2}\varepsilon k_0)^{1/2}$ the distance between secondary emissions is given by $\Delta x = (\frac{1}{2}\varepsilon k_0)^{-1/2}$. Then the energy loss rate is equal to $2\hbar\, \Delta k_0/\Delta x$, which is

$$\frac{\mathrm{d}E_0}{\mathrm{d}x^1} = -\varepsilon E_0 \tag{3}$$

Finally, it is possible to find the correspondence between the parameter ε in the refractive index equation and the equation of geodesic deviation. For a light ray travelling along the x-axis in an inhomogeneous medium there is the general result that $\frac{\mathrm{d}^2 y}{\mathrm{d}x^2} = -\frac{1}{n}\frac{\mathrm{d}n}{\mathrm{d}y}$. Combining this with equation (1) and the equation for geodesic deviation[5] we find

$$\varepsilon^2 = -(R^2_{\beta 2\delta} + R^3_{\beta 3\delta})\frac{\mathrm{d}x^\beta}{\mathrm{d}\tau}\frac{\mathrm{d}x^\delta}{\mathrm{d}\tau} \tag{4}$$

with $\beta, \delta = 0, 1, 2, 3$ where $R^\alpha_{\beta\gamma\delta}$ is the Riemann–Christoffel curvature tensor, and the distance is now measured by the more general affine parameter τ.

Evaluation of ε is readily done in the Post-newtonian approximation to general relativity. In this approximation the metric tensor is diagonal with elements

$$g_{00} = -(1 + 2\phi + 2\phi^2)$$
$$g_{ii} = 1 - 2\phi \ (i = 1, 2, 3)$$

where ϕ is the newtonian gravitational potential. The result is

$$\varepsilon^2 = 2\nabla^2\phi - 2\phi\frac{\partial^2\phi}{(\partial x^1)^2} + 2\sum_{i=1}^{3}\left(\frac{\partial\phi}{\partial x^1}\right)^2 \tag{5}$$

with the coordinate x^1 being used as the affine parameter. This gives ε the dimensions of inverse length. For a system of uniform density ρ we can use Poisson's equation to get from equations (3) and (5)

$$\frac{1}{E_0}\frac{\mathrm{d}E_0}{\mathrm{d}x} = -\left(\frac{8\pi G\rho}{c^2}\right)^{1/2} \tag{6}$$

where the replacement $x^1 \to x$ has been made. Near a single mass like the sun we have $\phi = -\frac{GM}{c^2 r}$ giving

$$\frac{1}{E}\frac{\mathrm{d}E}{\mathrm{d}x} = -\frac{2GM}{c^2 r^2}\left(1 - \frac{3x^2}{2r^2}\right)^{1/2} \tag{7}$$

Note that equation (6) comes from the first term in equation (5) which is linear in ϕ whereas equation (7) comes from the other terms that are quadratic in ϕ.

As the energy loss rate predicted by equation (6) is proportional to the photon energy the observed frequency shift cannot be distinguished from a doppler shift. It is of interest to determine whether equation (6) with the density of the intergalactic medium can account for the Hubble redshift in a static universe. For a density of n hydrogen atoms m^{-3} the predicted redshift is

$$z = (\lambda - \lambda_0)/\lambda_0 = \mathrm{e}^{-Hx} - 1 \simeq Hx \tag{8}$$

where $H = 5.59 \times 10^{-27} n^{1/2} \mathrm{m}^{-1}$. In more familiar units this is $51.7 n^{1/2}$ km s^{-1} (Mpc)$^{-1}$. Comparison with the conventional value[6] of 55 km s^{-1} (Mpc)$^{-1}$ shows that this theory can explain the Hubble redshift if $n \simeq 1\ \mathrm{m}^{-3}$.

The most direct measurement of the density of the intergalactic plasma has been made by Field and Perrenod[7] who find that the background X-ray observations come from a fully ionised plasma at a temperature of 10^8–10^9 K and a density of $n = 1.6C^{-1/2}\ \mathrm{m}^{-3}$, where $C = \langle n^2\rangle/\langle n\rangle^2$ is a clumping factor. However, this result was obtained with an expanding universe, where the density at past epochs was greater than this value. For a static universe of constant density (but still allowing an energy-loss with distance) a reanalysis of the same data gives $T = 1.3 \times 10^9$ K and $n = 2.4C^{-4/3}\ \mathrm{m}^{-3}$, which gives a Hubble value of $80C^{-2/3}$ km s^{-1} (Mpc)$^{-1}$. Unfortunately there is no direct evidence as to the value of the clumping factor C that would give a direct estimate of the Hubble constant. For this density the conventional value of 55 km s^{-1} (Mpc)$^{-1}$ requires that $C = 1.8$ and that there is an upper limit to the Hubble constant of 80 km s^{-1} (Mpc)$^{-1}$.

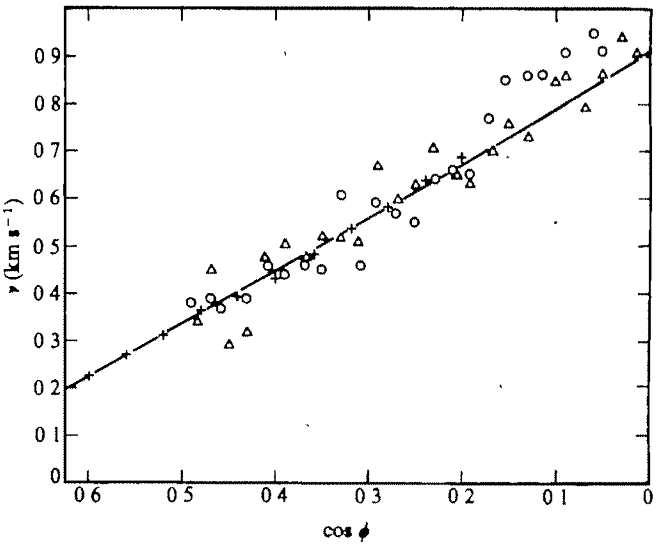

Fig. 1 The predicted (solid line) and observed solar limb effect. The redshift, expressed as a velocity is plotted against cos (ϕ) where ϕ is the angle between the line of sight and the solar radius. The solid line is the predicted shift from equation 9. The experimental results are: + Plaskett[10]; △, Adam[8]; and ○, Higgs[9]. All experimental results have been corrected for an apparent radial velocity of 0.39 km s^{-1} (ref. 11).

As this is only a plausibility argument several important corrections have been omitted. The first is that the cosmological curvature has been ignored. This could increase H by ~15%. The second is that relativistic corrections to the Gaunt factor (used in calculating the X-ray emission intensity) have been omitted. Finally the assumption of an iso-thermal intergalactic plasma has been made in spite of possible density variations. Nevertheless, I suggest that the Hubble redshift could be the observed result of a photon–gravitational interaction.

Careful measurements of the Fraunhofer lines wavelengths show a small but systematic redshift across the solar disk reaching a maximum on the limb. The effect can test the validity of the second order terms in the energy loss as given by equation 7. If ϕ is the angle between the line of sight and the solar radius, the expected redshift (expressed as an equivalent doppler velocity) is given by

$$v(\phi) = \frac{2GM}{Rc\sin(\phi)}\int_{\phi_0}^{\phi}\left(1 - \tfrac{3}{2}\cos^2(\phi)\right)^{1/2}\mathrm{d}\phi \tag{9}$$

where $\cos^2(\phi_0) = 2/3$. The effect is zero for $\phi < \phi_0$ and then increases monotonically to a value of 0.911 km s^{-1} at the limb. Equation (9) is plotted in Fig. 1 together with the observations of Adam[8], Higgs[9] and Plaskett[10]. All the observations have been corrected for a radial velocity of 0.39 km s^{-1} (as determined by Plaskett[11]). There is excellent agreement with the r.m.s. residuals from Plaskett's data being 0.012 km s^{-1} and for each of the others 0.05 km s^{-1}.

Integrated over the whole disk the average equivalent redshift velocity is 0.191 km s^{-1} in addition to the normal Einstein gravitational value of 0.634 km s^{-1}. Limb darkening alters this to 0.153 km s^{-1} (from Allen[12], with $u_2 = 0.84$, $v_2 = -0.20$). For other stars with similar limb darkening the equivalent redshift velocity is 0.787 m/r km s^{-1} where m and r are measured in solar units. This value is not inconsistent with observations of white dwarfs and may help to resolve some disagreements between gravitational radii and those estimated from model atmospheres.

This theory clearly predicts that if the line of sight to a radio source or a spacecraft passes near the Sun there will be a substantial redshift in the observed frequency. Observations[13] of Taurus A at 1,420 Mhz with an impact parameter of $5R_{\odot}$ suggest a possible shift of ~120 Hz, whereas observations[14] of W28 at 1,612 Mhz showed no shift. The most precise results[15,16] are observations of the Pioneer-6 spacecraft at 2,292 MHz. A consistent and unexplained redshift was observed as the spacecraft went behind the Sun, being 120 Hz at $4R_{\odot}$. The anomaly is that this theory predicts a shifts of 3,500 Hz in this case and shifts of ~2,000 Hz for the radio sources.

The explanation of the discrepancy possibly lies in an inhibition effect due to the presence of the solar corona. Efficient emission of radiation requires phase coherence of the emitting mechanism over a distance of the same size as the emitted wavelength. In this case the distance required is of order $k^{-1} = (\frac{1}{2}\varepsilon k_0)^{-1/2}$. It is convenient to think of the secondary radiation being produced by an interaction between the primary photon and a gravitational wave. However, the presence of a plasma, in this case the solar corona, alters the velocity of the photon but not that of the gravitational wave. If the primary photon has a frequency low enough to suffer a relative phase shift of π radians over the emission distance the rate of secondary emission will be decreased subtantially. For a plasma this critical frequency, ω_c, is given by $\omega_c = (2\omega_p^4/\varepsilon c)^{1/3}$ where ω_p is the circular plasma frequency. Typical values for the solar corona are $\omega_o = 1.0\times10^{11}$ Hz at 3 R_0 and $2.3\times10_{10}$ Hz at 10 R_0, showing that the critical frequency is well above the observational frequencies; thus strong inhibition of the interaction seems likely.

This is not the first proposal that relates the redshift to the effect of mass or gravitational fields on photons. Furth[17] derived an expression for frequency shift proportional to curvature by analogy with an electron in a circular orbit. However, he considered curvature in three spatial dimensions only and suggested that the energy loss went into gravitational waves. Stephenson[18] proposed that there would be a redshift due to absorption or shielding of gravitational flux. Gillieson[19] used a mechanical model for the photon to explain the observed limb effect. He arrived at a redshift proportional to M/r. A similar dependence was predicted by Proisy[20] whereas Sadeh, Knowles and Au[13] prefer M/r^2. Mo and Pappas[21] have treated the propagation of a classical electromagnetic wave in a Schwarzchild field which is treated as an equivalent medium. They find amplitude variations proportional to $\exp(M/r)$. However, none of these authors gives theory as widely applicable as the one presented here, nor do they consider the production of low energy photons as a result of the energy loss.

I argued previously[22] that the angular momentum and linear momentum cannot both remain invariant in curved space-time. The method used a classical description of the momenta as volume integrals of the Poynting vector and treated curved space-time as an inhomogeneous refractive medium. Although the predicted energy loss for a photon passing near the Sun is similar to equation (7) the over-simplified treatment of curved space-time makes the earlier results inaccurate.

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Observations of solar oscillations with periods of 160 minutes

OSCILLATIONS of the Sun with a period near 160 min have been reported by Severny *et al.*[1] and further described by Kotov *et al.*[2]. The presence of such oscillations would lead to a significant increase in our knowledge of solar physics. The reported amplitude is of the order of 1 ms^{-1} which is near the limit of observing technology. Observations described here at the Stanford Solar Observatory from 1975 to the present seem to support the reports from the Crimean Astrophysical Observatory[1,2].

At Stanford the relative velocity between a central circular area of radius $0.5R_{\odot}$ on the solar disk and most of the remaining area of the solar disk is measured. An optical scheme similar to that described in refs 1 and 2 is used and is described in detail by Dittmer[3].

Light from the central portion of the solar disk is passed through a right circular polariser and light from the outer portion through a left circular polariser. The solar line Fe I λ5124 Å is observed and a Babcock magnetograph is used to measure the wavelength separation between left and right circularly polarised light, that is the difference between the velocity of the central part of the solar disk and the outer portion.

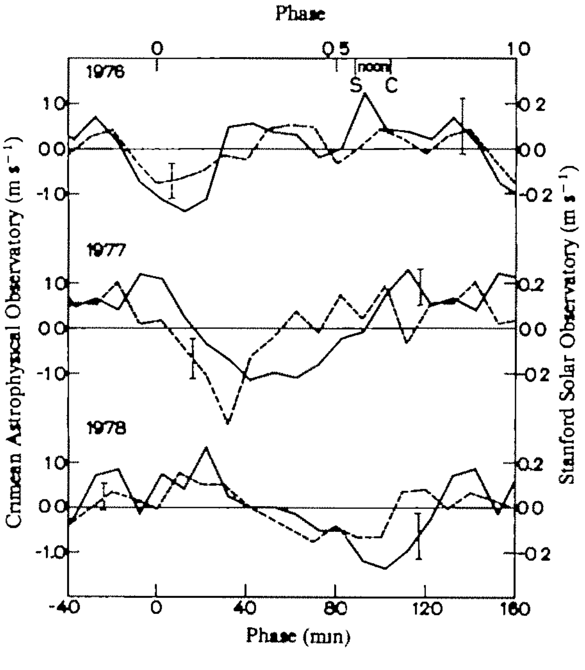

Fig. 1 Superposed epoch analyses with a period of 160 min for the observations during individual years at the Stanford Solar Observatory (solid line) and at the Crimean Astrophysical Observatory (dashed line) of the difference in velocity between the central portion of the solar disk and the outer portion.

Table 1 Summary of Stanford and Crimean observations

Year	Observatory	Dates	Days	Hours	Harmonic amplitude	Phase (min)
1974	Crimea	4 August– 9 October	14	76	1.43	−22
1975	Crimea	28 June– 14 October	28	157	0.89	−14
1976	Crimea	26 April– 15 October	32	161	0.36	8
	Stanford	1 July– 15 September	38	178	0.14	10
1977	Crimea	11 March– 24 October	59	280	0.78	32
	Stanford	6 April– 30 July	43	180	0.21	57
1978	Crimea	22 April– 20 August	60	361	0.47	82
	Stanford	7 June– 21 August	25	130	0.17	94

The fact that the period of 160 min is exactly one-ninth of a day requires that extreme caution be used in asserting that the observed oscillations are solar rather than terrestrial. Our observations considered as a continuous dataset lasting several months (or years) have considerable power at a period of 24 h because we do not observe at night and because there are drifts in the zero level through the day. At Stanford drifts in the guiding system are found to cause typically a 20 ms^{-1} drift in the signal baseline through a day. These drifts are removed by a parabolic fit. We describe here a superposed epoch analysis of the observations using a period of 160 min. This is equivalent to applying a filter that admits power corresponding to this period so that even if we have only noise input we will expect to receive a signal in the superposed epoch analysis. The comparison of observations between Stanford and the Crimea then rests largely on the relative phase of the observed oscillations at the two sites. If we both are observing a solar phenomenon we should expect to have the same phase within observational error, while if we are observing terrestrial phenomena or simply noise we should not expect phase agreement. During each of the years 1976, 1977 and 1978 the phase at the two observatories has been nearly the same, which supports the solar origin of the oscillations first reported at the Crimean Astrophysical Observatory[1,2]. Table 1 gives the observing schedule during each year and the observed phase.

Figure 1 shows a superposed epoch analysis with period 160 min of the observations during each year at the Stanford Solar Observatory (solid line) and at the Crimean Astrophysical Observatory (dashed line). Negative velocity represents expansion of the Sun (motion of the central part of the disk towards the observer, note that this is the opposite of the convention used in previous Crimean publications on this subject) and the error bars are twice the standard error of the means. The zero phase in Fig. 1 is at 0 h UT.

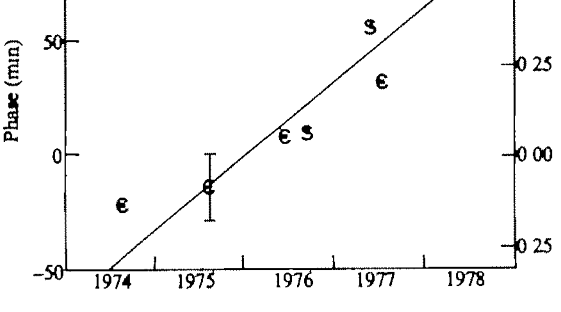

Fig. 2 Time of maximum expansion in minutes from 0 h UT for Stanford Observations (S) and Crimean Observations (C). The phase is computed from a harmonic analysis. The error bar shows the typical uncertainty in phase.

Figure 1 shows that in each of the three years the phase at the two observatories is nearly the same. Figure 1 also shows changes in amplitude from year to year that are about the same for both observatories. On a time scale of months there also seems to be similar changes in amplitude at the two observatories, but due to the small amount of simultaneous observations (due to weather and observing schedules) the significance and origin of these variations is still uncertain.

We compute based on instrumental parameters (the size of the portion of the solar disk that is compared with the central portion of the disk) that the lowest mode of solar oscillation would be observed at the Crimea with twice the amplitude of the Stanford signal. The remaining factor of about two shown in Fig. 1 between the amplitude of oscillations observed at the Crimea and at Stanford is not understood.

Figure 1 shows an apparent drift in phase from one year to the next. A drift in phase computed with a fixed period is the same as an error in the period. This suggests that 160.0 min may not be exactly the correct period. Figure 2 shows the phase for all the yearly sets of observations available from both observatories. The fact that the points from both observatories tend to fall nearly on a straight line suggests that both sets of observations show solar motions. From the slope of a best fit line we compute a drift in phase of about 33 min per yr corresponding to a period of about 160.01 min.

An unknown terrestrial effect related to the time of local noon could yield an agreement in phase between the two observatories if they were separated in longitude by an integral number of 360°/9 intervals, as we are dealing with a period of one-ninth of a day. In fact the two observatories are separated by 3.9 such intervals, such that Stanford would have a phase 16 min earlier than the Crimea. In the three years in which we have observed, Stanford tends to have a phase later than the Crimea.

The apparent agreement in phase between the two observatories as well as the fact that the period seems to differ slightly from exactly one-ninth of a day tends to support the interpretation that we are observing solar oscillations. The dependence of the amplitude of the oscillations on the phase of solar rotation recently observed in the Crimea[4] is also encouraging in this respect. Subject to the results of further years observations our present working hypothesis is that the oscillations are solar and that we can proceed towards further study of the modes involved and of the implications for our understanding of the Sun's interior.

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Photoelectrocatalytic reduction of carbon dioxide in aqueous suspensions of semiconductor powders

We report here the photoelectrocatalytic reduction of carbon dioxide to form organic compounds such as formic acid, formaldeyde, methyl alcohol and methane, in the presence of photosensitive semiconductor powders suspended in water as catalysts. Photocatalytic reaction kinetics were elucidated by reference to the theory of charge transfer at photoexcited semiconductors.

Heterogeneous photocatalytic reactions have recently been used to synthesise chemical compounds, thereby converting solar energy to chemical energy[1–4]. Schrauzer and Guth have reported the photocatalytic reduction of nitrogen on a catalyst of wet TiO_2 powder. Oxygen and hydrogen were formed through the decomposition of water, and ammonia and/or hydrazine were also obtained through the photo-assisted synthesis of nitrogen and water on the TiO_2 catalyst[3]. Semiconductor photoelectrodes have been much investigated, with emphasis on electrochemical photocells converting solar energy to chemical and/or electrical energy[5,6]. Photogenerated carriers (electrons and holes) in the space charge layer of a semiconductor electrode can transfer to the reactants in an electrolyte solution, and cause the photoassisted decomposition or synthesis of chemical compounds[7,8]. Semiconductor catalysts may be assumed to undergo charge transfer across a solid–liquid junction similar to that in semiconductor electrodes; that is, charge transfer between semiconductor and redox reagents in solution would take the form of a conduction band and a valence band. Hemminger *et al.* have reported that methane was formed through the photosynthetic reaction of gas phase water and carbon dioxide molecules adsorbed onto strontium titanate surfaces in contact with platinum foil[9]. Recently Halmann has reported that carbon dioxide in an aqueous solution was reduced to formic acid at the p-type GaP photocathode in the electrochemical photocell with a fairly high conversion efficiency[10].

In this study we attempted the photocatalytic reduction of carbon dioxide on various semiconductor photo-catalysts such as WO_3, TiO_2, ZnO, CdS, GaP, and SiC (99.5–99.9999% purity). In each case, semiconductor powder (200–400 mesh) 1.0–2.0 g was suspended in the 100 ml of purified water in a glass cell. Highly purified carbon dioxide gas was bubbled at 3 litres per min through a water-suspended catalyst shaken by a magnetic stirrer. The suspensions were illuminated, through a quartz window in the glass cell, by a 500 W Xe lamp or a 500 W high pressure mercury arc lamp. Illumination wavelengths were selected with cutoff filters. Products of the photocatalytic reactions were analysed by gas chromatography (Hitachi 163) using polyethyleneglycol (PEG) 200 and molecular sieve as column.

Table 1 Summary of yields of reduced products of carbon dioxide on various semiconductor catalysts

Catalysts (1.0 g/100 ml water)	Illumination period (h)	Yields of products HCHO ($\times 10^{-3}$ M)	CH$_3$OH ($\times 10^{-4}$ M)
TiO_2	7.0	1.1	2.3
TiO_2	14.0	1 8	7.8
TiO_2	30.0	1.8	14 6
TiO_2^*	7.0	0.0	0.0
ZnO	7.0	1.2	3.5
CdS	7.0	2.0	11.7
GaP	7.0	1.0	11.0
SiC	7.0	1.0	53.5
WO_3	7.0	0.0	0.0
Electrodes (−1.5 V vs SCE)	**Amount of charges (C)**	**Yields of products HCHO ($\times 10^{-3}$ M)**	**CH$_3$OH ($\times 10^{-4}$ M)**
TiO_2†	108	1.7	11.7
TiO_2‡	180	2.8	56 7
p-GaP§	240	3.6	73.0

* TiO_2 suspension was illuminated with light of wavelengths longer than 500 nm.

† TiO_2 electrode in 50 mM H_2SO_4 aqueous solution was not illuminated.

‡ TiO_2 electrode in 500 mM H_2SO_4 aqueous solution was not illuminated.

§ GaP electrode in 500 mM H_2SO_4 aqueous solution was illuminated with light of wavelengths shorter than 500 nm.

Formic acid, formaldehyde, and methyl alcohol were produced through the photocatalytic reduction of carbon dioxide in aqueous suspensions of semiconductors such as TiO_2, ZnO, CdS, GaP and SiC, when the suspensions were illuminated with light which was absorbable for the catalyst. A trace of methane was also detected in the gaseous products, which were trapped with liquid nitrogen refrigerant. Results of quantitative analysis of yields of reduced compounds from photocatalytic reduction of carbon dioxide in aqueous suspensions are given in Table 1. When the experiments were carried out in the dark or under illumination with a light not absorbed by semiconductor catalysts, no reduced products of carbon dioxide were detected with any of the semiconductor catalysts tested; as an example the results with TiO_2 are shown in Table 1.

The photocatalytic reactions are considered to follow the schemes.

$$\text{catalyst} + h\nu \longrightarrow \text{catalyst}^*(e^-_{\text{cond}} + p^+_{\text{val}})$$

for an oxidation

$$H_2O + 2p^+_{\text{val}} \longrightarrow 1/2O_2 + 2H^+$$

for reductions

$$CO_2(aq) + 2H^+ + 2e^-_{\text{cond}} \longrightarrow HCOOH$$
$$HCOOH + 2H^+ + 2e^-_{\text{cond}} \longrightarrow HCHO + H_2O$$
$$HCHO + 2H^+ + 2e^-_{\text{cond}} \longrightarrow CH_3OH$$
$$CH_3OH + 2H^+ + 2e^-_{\text{cond}} \longrightarrow CH_4 + H_2O$$

where e^-_{cond} and p^+_{val} denote an electron in the conduction band and a positive hole in the valence band, respectively.

Reduced compounds such as formaldehyde and methyl alcohol were best produced on SiC semiconductor catalyst after

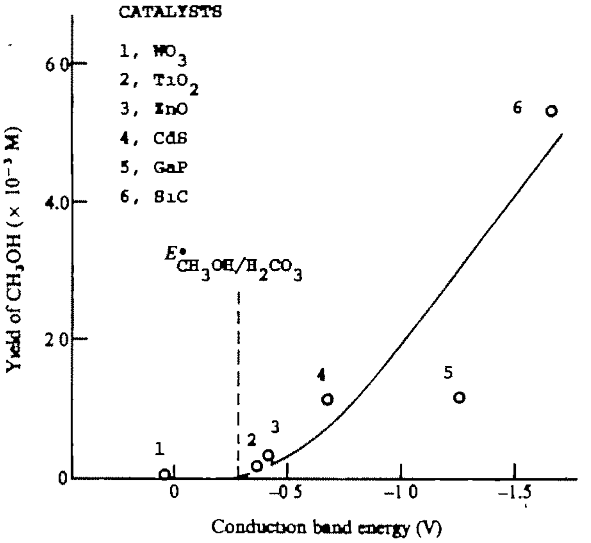

Fig. 1 Correlation between the yield of CH_3OH and the conduction bands of semiconductor catalysts. Dashed line denotes the standard redox potential of a CH_3OH/H_2CO_3 couple. Potentials are expressed against the normal hydrogen electrode (NHE).

illumination for 7 h, as shown in Table 1. Figure 1 shows the yields of methyl alcohol by photocatalytic reduction of carbon dioxide as a function of the energy levels of the conduction band of the semiconductor catalysts. The yields of methyl alcohol increase as the conduction band becomes more negative with respect to the redox potential of H_2CO_3/CH_3OH, whereas methyl alcohol was not produced in the presence of WO_3 catalyst which has a conduction band more positive than the redox potential of H_2CO_3/CH_3OH.

In experiments with semiconductor electrodes, carbon dioxide was reduced electrolytically to formic acid, formaldehyde, methyl alcohol and methane at the unilluminated TiO_2 electrode or at the illuminated p-type GaP electrode when both semiconductor electrodes were polarised at a potential of −1.5 V against a saturated calomel electrode for 2 h (Table 1). Thus electrons in the conduction band of semiconductor electrodes are able to reduce carbon dioxide in an aqueous solution.

It has been suggested that at semiconductor electrodes, the charge transfer rates between photogenerated carriers in semiconductors and the solution species depend on the correlation of energy levels between the semiconductor and the redox agents in the solution[11-13]. That is, the solution species of redox potential the more positive with respect to the conduction band level is better reduced at semiconductors. This hypothesis is illustrated in Fig. 2 which shows the energy correlation at the semiconductor–solution junction. When estimating charge transfer it is assumed that for semiconductor catalysts, as for semiconductor electrodes, photo-excited electrons in the more negative conduction band have the greater ability to reduce carbon dioxide in the solution.

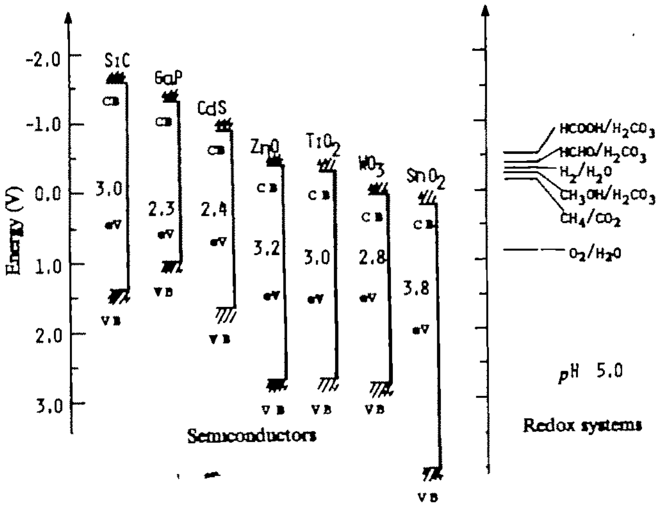

Fig. 2 A schematic illustration of the energy correlation between semiconductor catalysts and redox couples in water. CB and VB denote a conduction band and a valence band, respectively.

Thus the photocatalytic reaction on a semiconductor catalyst appears to take place through the action of the photogenerated carriers (electrons and holes) acting as components of a local cell—reduction by electrons occurring in a conduction band and oxidation by holes in a valence band. The estimated quantum yields of production for photocatalytic reduction of carbon dioxide were ~5.0×10^{-4} for HCHO and 1.9×10^{-4} for CH_3OH with a TiO_2 catalyst, and 5.0×10^{-4} for HCHO and 4.5×10^{-3} for CH_3OH with SiC as catalyst, measured against the absorbable incident photons. We are now investigating the detailed dynamics of the photocatalytic reaction on the semiconductor catalysts.

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Highly efficient quantum conversion at chlorophyll *a*–lecithin mixed monolayer coated electrodes

THE design of solar conversion systems based on the photosynthetic primary reactions has attracted much attention recently. For instance, photoelectrolysis at a chlorophyll (Chl) *a*–water aggregate coated platinum electrode as a photocathode has been studied by Fong *et al.*[1-3], and simulation of the photoinduced electron transfer across the thylakoid membrane as well as of the biological ordered structure in a chloroplast has been attempted by Tien *et al.*[4,5] using a Chl *a*–containing bilayer lipid membrane (BLM). We have already attempted to combine these different approaches[6] by using a Chl *a* monolayer coated SnO_2 transparent electrode as a photoanode, and a maximum photocurrent quantum efficiency of 12–16% was attained with Chl *a*–stearic acid mixed monolayer systems. In that case, however, a decrease of the quantum efficiency was observed at Chl *a*–stearic acid molar ratios <1.0; this might be due to the formation of pheophytin *a* and/or to a possible heterogeneity of the mixed monolayers. We describe here how we have overcome such problems by using a phospholipid instead of the fatty acid as a

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diluent for a Chl *a* monolayer. The hydrophobic interactions between Chl *a* and phospholipids have been well investigated both *in vivo* and *in vitro*, and the physical stability and photochemical activity of Chl *a*–synthetic lipid mixed layers were also confirmed in the BLM study[7].

Chl *a* was prepared from spinach as previously reported[6]. Synthetic L-α-dipalmitoyl lecithin (DPL, Sigma) was used as a phospholipid without further purification. An SnO_2 optically transparent electrode (OTE)[6] was used as a substrate of the monolayer deposition. The mixed monolayers of Chl *a* and DPL were prepared by spreading 2×10^{-4} M benzene solutions of Chl *a* and DPL in various molar ratios onto 10^{-3} M phosphate buffer solution (pH 7.5–8.0) in a Langmuir trough. A single layer of the mixed monolayer formed on the aqueous surface was deposited onto the SnO_2 OTE under the constant surface pressure of 20 dyn cm^{-1}. The electrochemical measurement setup was as reported previously[6]. The electrolyte solution used consisted of 0.1 M Na_2SO_4, 0.025 M phosphate buffer (pH 6.9), and 0.05 M hydroquinone as a reducing agent, and was flushed with nitrogen throughout the experiments. Monochromatic light from a 500-W Xe-arc lamp irradiated the monolayer coated SnO_2 OTE and the photocurrent was measured by a Keithley picoammeter (Model 417) in a potentiostatic condition of +0.1 V *versus* saturated calomel electrode (SCE).

The surface pressure–area (*F*–*A*) characteristic of Chl *a*–DPL mixed monolayer is compared in Fig. 1 with that of pure Chl *a*[6]. The stability of the mixed monolayer, even at pressures higher than 24 dyn cm^{-1} (the collapse pressure of Chl *a* monolayer), implies that the present Chl *a*–DPL mixed monolayer could form a homogeneous two-dimensional solution. This homogeneity can be also supported by the observed change in the absorption property of the mixed monolayer following the dilution of Chl *a* content. On decreasing the molar ratio of Chl *a*–DPL from 1:0 to 1:99, the peak position of the red absorption band was found to shift gradually from 675 nm due to an aggregated state of Chl *a* towards 665 nm due to the monomeric state, accompanying the decrease in absorbance in proportion to the surface concentration of Chl *a*. These physical and spectral properties indicate the formation of homogenously mixed monolayer as reported for other Chl *a*–diluent systems[8,9].

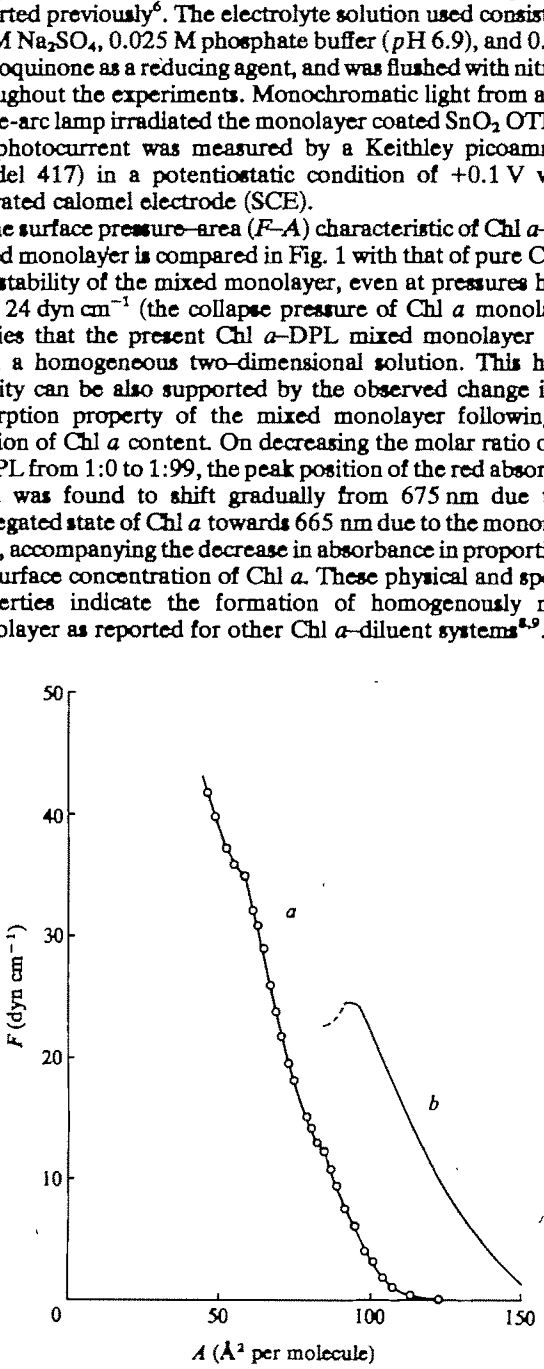

Fig. 1 *F*–*A* characteristics of a Chl *a*–DPL (1:1) mixed monolayer and a Chl *a* monolayer: *a*, Chl *a*–DpL (1:1) mixed monolayer; *b*, pure Chl *a* monolayer. Aqueous phase, 10^{-3} M phosphate buffer (pH 7.5); air atmosphere at 25 °C.

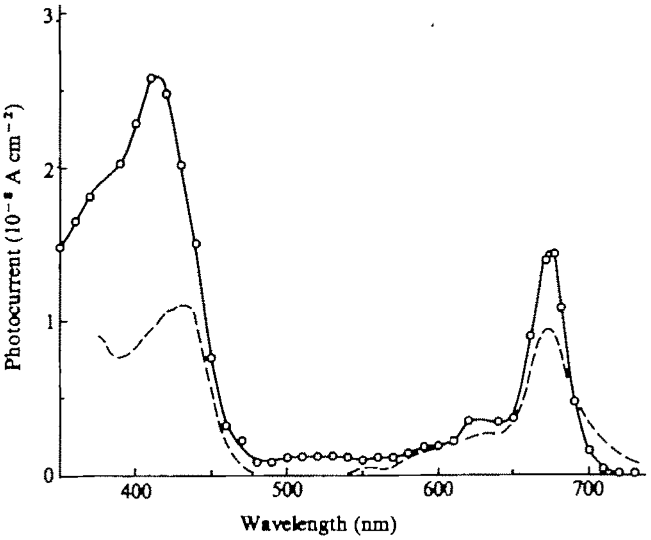

Fig. 2 Photocurrent spectrum at SnO_2 OTE deposited with mixed monolayer of Chl *a*–DPL (1:9). Dashed curve indicates the absorption spectrum of the mixed monolayer at the OTE-electrolyte interface. Composition of the electrolyte solution, 0.05 M hydroquinone, 0.1 M Na_2SO_4, and 0.025 M phosphate buffer (pH 6.9); electrode potential 0.1 V *versus* SCE; incident flux of monochromatic light, 2×10^{14} photons s^{-1} cm^{-2}.

When a Chl *a*–DPL mixed monolayer was irradiated in the electrochemical cell, an anodic photocurrent was always obtained in accordance with the electron injection from excited Chl *a* into the conduction band of SnO_2. A typical photocurrent spectrum, corrected for the slight background photocurrent of SnO_2, for the mixed monolayer of molar ratio Chl *a*–DPL of 1:9 is shown in Fig. 2. The magnitude of photocurrent corresponds to a constant incident flux of 2×10^{14} photons s^{-1} cm^{-2} at each wavelength. The photocurrent spectra coincided well with the absorption spectra of mixed monolayers at OTE–electrolyte interface. The quantum efficiencies of photocurrents were determined in terms of electrons flowing per absorbed photon, the latter could be estimated from the incident photon flux measured by chemical actinometry and from the monolayer absorbance at an OTE–electrolyte interface. The photocurrent quantum efficiencies obtained with mixed monolayers at different molar ratios are listed in Table 1, together with the calculated mean intermolecular distances between Chl *a* molecules. As the Chl *a*–Chl *a* intermolecular distance increases, the photocurrent efficiency also tends to increase, with a hypso- and bathochromic shift of the photocurrent peak positions in the red and blue regions, respectively. Such an enhancement of the quantum efficiency is attributable to the suppression of intermolecular energy transfer between Chl *a* molecules by the increase in its intermolecular distance, because the energy transfer inevitably accompanies an energy loss which eventually decrease the overall efficiency of electron injection from excited Chl *a* to SnO_2. The concomitant shifts of photocurrent peak positions are compatible with the shifts in absorption peaks described above, and the absence of the characteristic peaks due to pheophytin *a* around 400–410 nm and 500–540 nm (ref. 10) in both photocurrent and absorption spectra of mixed monolayers indicates that the pheophytin formation is effectively suppressed in the present systems; a similar stability against the pheophytinisation was recently investigated by Iriyama (ref. 11 and personal communication).

The maximum efficiency of 25±5% obtained in this work at neutral pH is relatively large, and almost twice that attained with the Chl *a*-stearic acid system[6]. Moreover, an examination of the pH dependence of the photocurrent showed that the quantum efficiency could reach 30±5% when the experiment was done in the electrolyte solution at $p\text{H} \sim 5$. These values are sufficiently larger than the quantum conversion efficiencies reported ($10^{-3} \sim 10^{-1}$) (refs 3, 4, 12–14) with other solar conversion cells using Chl *a* as a photoreceptor.

Meilanov *et al.*[15] in their attempt to verify the semiconductor model of photosynthesis, found a relatively high quantum efficiency (12–20%) for bulk photoconductive effects in metal-sandwiched dry Chl layers (0.3–2 μm thick) across which a high voltage (30–250 V) was applied. Our system is essentially different in that the photocurrent is produced by a direct electronic coupling between an excited Chl and the conduction band of SnO_2, not by an accelerating effect of the electric field across the Chl layer.

An extension of the present experiments to the construction of biomimetic membranes on SnO_2 OTE by incorporating other photosynthetically important compounds into the Chl *a*–DPL mixed monolayer would be highly promising in view of the *in vitro* simulation of the photosystem II in plant photosynthesis.

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Enhanced CO_2 greenhouse to compensate for reduced solar luminosity on early Earth

CURRENT models for the evolution of the Sun require an increase in solar luminosity by 25% since the formation of the Solar System[1]. Such an increase in the solar constant should have profound effects on the terrestrial climate, but there is no evidence from the fossil record of a corresponding change in the Earth's global mean temperature[2]. This apparent conflict cannot be explained by the apparent inability of solar models to account for the low observed neutrino flux[3]. Even models that are forced to fit the neutrino data require a similar increase in the solar luminosity. As Newman and Rood[1] state: "a faint young Sun is one of the most unavoidable consequences of stellar structure considerations". We discuss here whether CO_2–H_2O in a weakly reducing atmosphere could have caused this change in the early Earth's temperature by the so-called greenhouse effect.

Sagan and Mullen[4] suggested that the solution must lie in a more efficient atmospheric greenhouse effect on Earth during the period when the solar luminosity was low. To increase the greenhouse effect, it is necessary to change the atmospheric composition, or density, or both. Sagan and Mullen[4] postulated that the atmosphere was reducing during the early history of the Earth and found that a small amount of ammonia in the atmosphere (mixing ratio 10^{-5}–10^{-6}) would provide the necessary opacity to outgoing infrared (IR) radiation to increase the mean surface temperature. In this model, the transition from reducing to oxidising conditions with the replacement of the NH_3–H_2O greenhouse by a CO_2–H_2O greenhouse comes about as a result of the development of widespread green plant photosynthesis 1,000–2,000 Myr ago. A similar route was invoked by Hart[5], who developed a more detailed model for the evolution of the atmosphere.

We agree that an enhanced greenhouse effect caused by a change in atmospheric composition is a good solution to the solar constant dilemma, but we think the NH_3–H_2O model is unrealistic. Current thinking about the early atmosphere of the Earth is shifting away from the traditional view that called for a highly reduced mixture of methane, ammonia and hydrogen, for reasons summarised by Walker[6]. More recent data have simply added strength to Walker's conclusions. As the noble gases on Mars show the same pattern of relative abundances as they do on Earth and in the 'planetary component' of the meteoritic gases, whatever fractionation process caused this pattern must have occurred before planet formation[7]. This being the case, the cosmic abundance ratio of 1.6×10^4 for H/Ne[8] gives the maximum amount of hydrogen available for a 'captured' primitive atmosphere. Using the present atmospheric partial pressure of Ne (12.7×10^{-6} bar), the maximum hydrogen partial pressure on the primitive Earth from this source was 10 mbar. As Walker[6] has pointed out, such an atmosphere would have dissipated in $<10^4$ yr. The partial pressure of ammonia in this atmosphere would have been comparable to that of neon, but its lifetime against photodissociation would have been short and it would have been out of equilibrium with crustal rocks.

Photochemical calculations by Kuhn and Atreya[9] indicate that ammonia would be irreversibly converted to N_2 on the primitive Earth in <40 yr if the mixing ratio were $\leq10^{-4}$. Eugster[10] has investigated the stability of ammonia on the primitive Earth and concluded that a mixture of N_2 and H_2 will predominate over ammonia in a normal crustal environment. Outgassing of ammonia after the last phase of accretion (or after differentiation, choosing either an inhomogeneous or homogeneous model for planet formation) seems precluded by the lack of free iron in the upper mantle[6]. As life must have begun after this phase in the planet's history, it seems reasonable to agree with Eugster[10] that any initial ammonia in the atmosphere "was oxidised to nitrogen long before the origin of life". Small amounts of methane and ammonia may have been transiently present in later times as a result of meteoritic and cometary impact, but lunar evidence indicates that bombardment rates must have been close to their present low values ~3,600 Myr ago[11].

In fact, the idea that the chemistry preceding the origin of life must have occurred in highly reducing conditions supported the traditional view of the composition of the early atmosphere[12]. But there have been sufficient demonstrations that pathways towards the formation of essential pre-life compounds can occur only in weakly reducing conditions for this argument to have lost its original force. For example, Abelson[13] was able to produce amino acids from the UV irradiation of a mixture of CO_2, CO, N_2, H_2O and just 1–10% H_2. This seems a much more reasonable composition for the post-accretion (or post-differentiation) atmosphere of the primitive Earth[6,10]. It is especially unlikely that the ammonia-containing mixture invoked by Sagan and Mullen[4] (or ammonia plus methane suggested by Hart[5]) would last for 2,000–3,000 Myr after formation of the planet. But can this weakly reducing atmosphere provide the required greenhouse effect? The answer depends on carbon dioxide and water vapour, as the other gases are ineffective absorbers of IR radiation.

We have tested this possibility by assuming that the concentration of CO_2 in the early atmosphere was much higher than the present value. We have coupled the evolutionary model for the CO_2 abundance used by Hart[5] with a radiative–convective CO_2-climate model. (We used the model as in Augustsson and

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Table 1 Predicted global surface temperature, T_s, throughout the evolution of the Earth's atmosphere

Time (10^9 yr BP)	S (W m^{-2})	P_{CO_2} (bar)	T_s (K)	
4.25	1,039	0.31	310	(284)
3.5	1,096	0.070	296	(284)
3.0	1,133	0.033	293	(284)
2.5	1,171	0.018	292	(286)
2.0	1,209	0.0086	290	(286)
1.5	1,247	0.0029	288	(286)
1.0	1,284	0.00065	286	(285)
0.5	1,322	0.00032	287	(287)
0	1,360	0.00032	290	(290)

The solar constant, S, is taken to be $S = 1{,}020$ W m^{-2} 4,500 Myr ago and is assumed to increase linearly with time. The CO_2 surface pressure, P_{CO_2}, is from Hart[5]. Temperatures listed in parentheses refer to deletion, within the model, of weak CO_2 bands at 9–10, 12 and 18 μm.

Ramanathan[14] except that we do not include the 7.6 μm pressure-induced band of CO_2 in the present analysis, because of the lack of band absorption data for the large amounts of CO_2 considered here.) Hart's model comprises a numerical simulation of the evolution of the Earth's atmosphere over its 4,500 Myr history, accounting for time-dependent outgassing, the Urey equilibrium, the onset of photosynthesis, and crude atmospheric chemistry to develop the time-history of the CO_2 concentration. Hart also presents NH_3 and CH_4 concentrations but we do not believe that these gases would persist within the early atmosphere. Moreover, even if CH_4 were present in the early atmosphere, this constitutes a very inefficient greenhouse gas. Quite obviously Hart's attempt contains many modelling uncertainties, but at least it produces a first estimate for the variability of atmospheric CO_2 over geological time.

Although Hart[5] has considered the greenhouse effect from enhanced CO_2, as well as other gases, he uses grey gas radiation modelling. But the realities of accounting for enhanced CO_2 require a more detailed radiation model[14,15]. Moreover, Hart has used a cloud radiation model which produces strong negative climate feedback, contrary to the findings of recent cloud-climate modelling studies[16,17]. This occurs as Hart relates global cloud amount to atmospheric water-vapour content, such that his cloud amount increases with increasing planetary surface temperature. But recent model predictions suggest that the reverse occurs (see ref. 16). Hart has additionally neglected the IR opacity of clouds. Thus, in his model, a warmer planet produces an enhanced planetary albedo due to increased cloudiness, but with no compensatory IR modification, and for this reason his model gives negative cloud feedback.

In the present study, we ignore climate-induced changes in cloud-cover fraction, following the suggestion that the cloud albedo and IR modifications are compensatory[17]. Moreover, we do not attempt to incorporate ice-albedo feedback, as the extent of the polar caps seems to depend on ocean advection and, in turn, the positions of the continents which have changed considerably over geological time[18]. Even without this effect, there are substantial uncertainties in modelling ice-albedo feedback[19]. Our model mostly predicts global temperatures greater than those at present; neglect of positive ice-albedo feedback will simply produce an underestimate of these temperatures. We further assume that the Earth's surface albedo is constant and equal to its present value, ignoring changes caused by mountain building and surface vegetation.

The results of our modelling are summarised in Table 1. We have simply assumed a total atmospheric pressure of 1 bar; the predicted global temperatures are not particularly sensitive to this assumption. The solar constant was taken to be 1,020 W m^{-2} (75% of the present value), 4,500 Myr ago.

The predicted global temperatures of Table 1 suggest that enhanced CO_2 concentrations could have been the sole mechanism for preventing glaciation of the Earth when there was reduced solar luminosity. Past global glaciation would be implied by climate models in the absence of an increased atmospheric greenhouse[20]. Within the confines of our model calculations, the Earth was never more than 4 K colder than at present. This, of course, does not account for glacial–interglacial oscillations which, on our time scales, comprise short-term climatic variability.

A detailed analysis of our model results revealed that some weak bands of CO_2, which have been neglected in previous studies of this problem[4,5], contribute significantly to the surface warming. These comprise two bands at 12 μm, two bands at 18 μm, and two bands in the 9–10 μm region. Predicted global surface temperatures, deleting these weak bands within the model calculation, are included in parentheses in Table 1. Clearly, these weak bands are important for large CO_2 concentrations. In this context, the neglect of the 7.6 μm band in the present model would produce an underestimate of the computed surface temperatures by about 5–10 K for CO_2 surface pressures of the order of 0.1–0.3 bar.

Our results are quite insensitive to events occurring from 4,500 to 4,250 Myr ago. For example, if there were an initial global glaciation during this time period, due possibly to limited outgassed CO_2, the CO_2 greenhouse effect at 4,250 Myr ago would be sufficient to melt the surface ice. To show this, taking the surface albedo for an ice-covered Earth to be 0.7, we estimate the corresponding global temperature 4,250 Myr ago to be 285 K, far too high for global glaciation to persist.

We conclude that there is no need for ammonia to be present in the early atmosphere to maintain moderate temperatures when the Sun's luminosity was low. Carbon dioxide alone is sufficient, provided the partial pressure of this gas approaches the values suggested by the evolutionary model of Hart[5]. In view of the huge amount of CO_2 that has been produced over geological time and our lack of knowledge about rates at which release and deposition occurred in primitive times, this does not seem an unwarranted assumption. In fact, more detailed calculations may permit us to place a limit on the amount of CO_2 that could ever have been present in the atmosphere at one time, as the full complement (some 70 bar) would have caused excessive warming of the Earth's surface. We are also pursuing applications to the atmosphere of Mars, where larger CO_2 abundances in primitive times might have provided a sufficient greenhouse effect to permit the flow of liquid water apparently required by the evidence for fluvial erosion on that planet's surface.

The transition to an oxidising atmosphere on Earth between 2,000 and 2,500 Myr ago, brought about by green-plant photosynthesis, is then not accompanied by a transition from an NH_3–H_2O to a CO_2–H_2O greenhouse, but rather represents a simple increase in free molecular oxygen with the concomitant disappearance of CO. The composition of the intermediate atmosphere, spanning ~2,000 Myr from the early escape of hydrogen to the onset of oxygen-producing photosynthesis, was probably very similar to the present atmospheres of Mars and Venus, two planets on which any living organisms (if present) apparently have not been able to influence atmospheric composition as they have on Earth[21,22].

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2,390 Myr Rb–Sr whole-rock for the Scourie dykes of north-west Scotland

THE Scourie dyke swarm intrudes the Lewisian Complex in north-west Scotland. The structural state of these basic dykes is used to subdivide the Lewisian into the pre-dyke 'Scourian' and post-dyke 'Laxfordian' complexes[1]. The ~2,700 Myr (refs 2–4) pyroxene-granulite facies Scourian gneisses of the Assynt area contain undeformed NW-trending dykes. Gneisses and dykes, which underwent the ~1,900 Myr (refs 5, 6) Laxfordian event, are now deformed and metamorphosed at amphibolite facies. Previous K–Ar measurements for the Scourie dykes in the Assynt area of the Lewisian gave a minimum age for dyke injection of 2,200 Myr (ref. 7). Younger K–Ar ages were thought to reflect later Laxfordian overprinting. I report here a Rb–Sr whole-rock isochron age of 2,390 ± 20 Myr (2σ) and an initial $^{87}Sr/^{86}Sr$ ratio of 0.7022 ± 0.0001 (2σ) for the Scourie dykes.

The Rb–Sr whole-rock method is in principle more suited to the problem of determining the emplacement age of the Scourie dykes. However, the range in Rb/Sr and measured $^{87}Sr/^{86}Sr$ ratios of the individual dykes is very small, and so any geological scatter in the data points will result in a large error in the 'fit of the isochron' and, hence, in the calculated age. Table 1 gives Rb–Sr whole-rock isotopic analyses for suites of samples from three Scourie dykes in the area of least post-2,700 Myr deformation and metamorphism at Scourie. The dykes at north and south Scourie Bay are fresh two-pyroxene dolerites[8], but the Kylesku Dyke contains amphibole replacement of pyroxene[6].

The ages and initial $^{87}Sr/^{86}Sr$ ratios of these three dykes, presented in Table 2, are all within 2 sigma error limits. Statistical tests (Student's *t*-test) show that the slopes of the isochrons are not significantly different at 95% confidence limits. There is no geological or petrological evidence to suggest that these dykes are not cogenetic or coeval. The 33 data points combined define an isochron (MSWD = 2.1) which gives an age of 2,390 ± 20 Myr (2σ) and an initial $^{87}Sr/^{86}Sr$ ratio of 0.7022 ± 0.0001 (2σ) (Fig. 1). This low initial $^{87}Sr/^{86}Sr$ ratio is within the range thought characteristic of rocks derived from upper mantle source regions at ~2,400 Myr (ref. 9). The age from this isochron is interpreted as being close in time to the primary igneous crystallisation of the dolerite dykes in the Scourie region. Combining the three sets of data has increased the range in Rb/Sr ratio and considerably reduced the calculated error on the time of emplacement. This low ±20 Myr uncertainty depends on the dykes being intruded at the same time and from the same source region. The combined Rb–Sr whole-rock age of 2,390 ± 20 Myr is considered the best estimate for the time of emplacement of the Scourie dykes investigated.

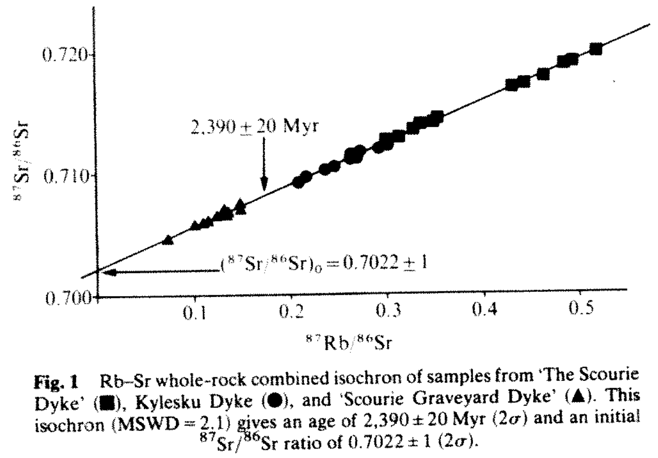

Fig. 1 Rb–Sr whole-rock combined isochron of samples from 'The Scourie Dyke' (■), Kylesku Dyke (●), and 'Scourie Graveyard Dyke' (▲). This isochron (MSWD = 2.1) gives an age of 2,390 ± 20 Myr (2σ) and an initial $^{87}Sr/^{86}Sr$ ratio of 0.7022 ± 1 (2σ).

Table 1 Rb–Sr whole-rock isotopic analyses for samples from three Scourie dykes

Sample no.	Rb/Sr (±2% ≮0.002)	Rb (p.p.m. ± 10%)	Sr (p.p.m. ± 10%)	$^{87}Sr/^{86}Sr$	$^{87}Rb/^{86}Sr$
'The Scourie Dyke', north Scourie Bay (GR 151455–144458)					
MC 64	0.116	16.2	139	0.71395 ± 8	0.336
MC 65	0.103	14.9	145	0.71265 ± 4	0.299
MC 66	0.168	25.0	148	0.71901 ± 6	0.488
MC 67	0.121	18.3	151	0.71432 ± 6	0.350
MC 68	0.115	17.2	151	0.71363 ± 6	0.331
MC 69	0.180	25.8	144	0.71997 ± 8	0.520
MC 70	0.170	25.5	149	0.71923 ± 4	0.492
MC 71	0.108	16.4	152	0.71295 ± 6	0.313
MC 72	0.148	22.7	153	0.71717 ± 6	0.429
MC 73	0.154	22.7	147	0.71731 ± 4	0.445
MC 74	0.161	24.3	151	0.71806 ± 4	0.466
MC 75	0.122	18.0	148	0.71440 ± 4	0.352
Kylesku Dyke (GR 234330)					
MC 76	0.0920	15.3	166	0.71149 ± 6	0.266
MC 77	0.1025	17.1	166	0.71221 ± 4	0.297
MC 78	0.0930	15.2	164	0.71131 ± 4	0.269
MC 79	0.0725	13.0	179	0.70926 ± 4	0.210
MC 80	0.1030	18.1	176	0.71246 ± 3	0.298
MC 81	0.0855	12.1	141	0.71074 ± 8	0.247
MC 82	0.0930	13.1	142	0.71163 ± 4	0.269
MC 83	0.0745	12.5	169	0.70980 ± 4	0.216
MC 84	0.0810	13.4	166	0.71038 ± 4	0.234
MC 86	0.0915	15.2	166	0.71149 ± 4	0.265
MC 87	0.0935	15.1	161	0.71168 ± 6	0.271
'Scourie Graveyard Dyke', south Scourie Bay (GR 148448)					
MC 13	0.0385	7.3	185	0.70600 ± 6	0.111
MC 14	0.0395	8.5	215	0.70594 ± 6	0.114
MC 15	0.0255	4.1	160	0.70458 ± 4	0.074
MC 16	0.0460	6.9	150	0.70683 ± 4	0.133
MC 17	0.0520	7.9	151	0.70708 ± 4	0.150
MC 18	0.0515	7.7	149	0.70757 ± 8	0.149
MC 19	0.0460	6.7	146	0.70696 ± 8	0.133
MC 20	0.0420	7.3	173	0.70641 ± 6	0.122
MC 21	0.0460	6.5	142	0.70665 ± 6	0.133
MC 22	0.0350	5.7	164	0.70569 ± 4	0.101

Rubidium and strontium were separated from the 3–5-kg samples by the standard techniques used in this laboratory[6,17]. Isotope analyses were made on a 12-inch solid-source mass spectrometer using single Ta filaments[18]. Sr blank was always better than 6 ng and Rb blank less than 2 ng. Rb/Sr ratios were determined by X-ray fluorescence using extended counting times (not less than 400 s) on both peaks and backgrounds. The precision on duplicate analyses was always better than 2% or ±0.002 (whichever is the greater) and were checked against isotope dilution analyses. The value of $1.42 \times 10^{-11}\ yr^{-1}$ was used for the ^{87}Rb decay constant[19]. All errors are quoted at the 2 sigma level.

The main Scourian event formed granulite facies gneisses and NE–SW-trending structures. A later phase of NW–SE folding and associated almandine–amphibolite metamorphism has been recognised in the Assynt area[10–12]. This younger Inverian[10] event was synchronous with or occurred immediately before the intrusion of the Scourie dykes. The 2,390 ± 20 Myr date for the emplacement of the Scourie dykes provides a minimum age for this Inverian event. This is significantly older than the 2,200 Myr age previously quoted for this event[7,10–16] and perhaps indicates that the Inverian was a late phase of deformation and retrogressive metamorphism in the major Scourian event.

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Table 2 Ages and initial $^{87}Sr/^{86}Sr$ ratios of three Scourie dykes

Locality	No. of data points	Age (Myr)	$(^{87}Sr/^{86}Sr)_0$	MSWD
North Scourie Bay	12	2,330 ± 60	0.7025 ± 3	1.4
Kylesku	11	2,380 ± 150	0.7023 ± 6	2.3
South Scourie Bay	10	2,580 ± 220	0.7018 ± 4	2.4
Mean (Fig. 1)	33	2,390 ± 20	0.7022 ± 1	2.1

The ~2,400 Myr age for the emplacement of the Scourie dyke swarm indicates that the Scourian tectonic episode had ceased by the beginning of the Proterozoic. This area of early continental crust then remained stable until the Laxfordian metamorphic and deformational event.

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Geomagnetic intensity in Athens between 2000 BC and AD 400

MEASUREMENTS of the intensity in Athens of the geomagnetic field between 2000 BC and AD 400 are reported here. The material from which the intensity was determined consisted primarily of sherds from the Agora excavations. Some sherds, and a whole skyphos, from the collection of the Ashmolean museum at Oxford were also sampled. The ancient intensity of the Earth's magnetic field was inferred from stepwise thermal demagnetisation. This is essentially the technique developed by Thellier[1], with the modifications described by Walton[2].

The magnetisation was measured with a Squid magnetometer which was essentially that described by Walton[2]. Of the alterations to the apparatus the major change was to move the heating oven and field coils to a position below the magnetometer. The manipulations of the specimen were accomplished pneumatically. These were controlled by a small computer which also processed the signal from the Squid, printing out the components of the magnetisation, **M**.

The laboratory field, $\mathbf{H}_z$, was applied in the vertical direction and, as described by Walton[2], an attempt was made to minimise the change in the vertical component of **M**, $\mathbf{M}_z$. In practice, the choice of field was within 10–20% of the correct value. Accordingly, values of $\mathbf{M}_z$ were obtained both with a laboratory field applied, $\mathbf{M}_z^H$, and with no field applied $\mathbf{M}_z^0$. The values of $\mathbf{M}_z^H$ were then plotted against $\mathbf{M}_z^0$ and the laboratory field corrected to give that field which would have produced a stationary value of $\mathbf{M}_z^H$. Values were also obtained for the transverse component, $\mathbf{M}_y^H$ and $\mathbf{M}_y^0$. The values of $\mathbf{M}_y^0$ were plotted against $\mathbf{M}_z^0$, and the slope of the resultant line yielded the angle between **M** and **H**. By dividing the corrected laboratory field, **H**, by the cos of this angle the estimated value of the ancient field was obtained.

Measurements were made, using the above procedure at temperatures which were nominally 200, 250, 300, 400, 500, and 600 °C (the higher temperatures were often omitted if the sample had already demagnetised sufficiently at lower temperatures).

In addition, values of $\mathbf{M}_y^H$ and $\mathbf{M}_z^H$ were obtained at intermediate temperatures. $\mathbf{M}_z^H$ was then plotted against $\mathbf{M}_y^H$. As no component of **H** was present in the transverse direction, $\mathbf{M}_y^H$ provided a measure of the degree to which the sample had demagnetised. Thus the plot of $\mathbf{M}_z^H$ against $\mathbf{M}_y^H$ could also be used to correct **H**. Both plots were used, and the value of **H** which was used was an average of the two.

Finally, the angle between $\mathbf{M}_y^0$ and some arbitrary direction in the horizontal plane was monitored. The aim of this procedure was to identify the presence of possible additional components of **M** introduced by reheating the pottery in antiquity.

Attic pottery is, on the whole, quite strongly magnetised, a magnetisation density of 10^{-3} e.m.u. cm^{-3} being typical. Thus in principle a correction for demagnetising fields due to sample shape should be applied. However, this correction would not amount to more than the difference between the value for a disk with the field applied along a diameter, or at right angles to it. As the thickness of the sample was approximately equal to the diameter this correction was negligible.

A potentially more serious problem arises from demagnetising effects due to the shape of the vessel itself affecting the moment produced during firing in antiquity. In an attempt to estimate the magnitude of this effect, samples were taken from the base, sides and handles of an Attic skyphos which had been fired between 440 and 425 BC. However, before the results could be assessed a more serious source of error had to be considered.

Rogers (personal communication) has found that pottery is anisotropic: it is easier to magnetise in a direction parallel to the plane of the pot, than it is at right angles. It is possible to correct for this effect if the anisotropy ratio—the ratio of the moment produced in the hard direction to that in the easy direction—is known. The angle between the easy plane of magnetisation and **H** is known, and if the angle between this plane and the ancient field $\mathbf{H}^A$ is known, these two angles can be deduced from the changes in direction of **M** which occur on magnetisation in the laboratory field. The derivation of this correction factor will be detailed elsewhere. It turns out that the correction is simplified considerably if the sample is orientated so that **H** is either parallel or antiparallel to the laboratory field.

The results obtained from the Attic skyphos were corrected in this way. The anisotropy ratio was adjusted to yield the best agreement between samples taken from the base and those from the side, this amounted to 0.9. For these samples, the shape correction, if any, would be less than the scatter in the data. Hence, no shape correction was made. Unfortunately, the anisotropy correction of the results for the handles proved impossible because of instrumental difficulties, and these had to be discarded.

Having done this it was impossible to identify any shape effect, except possibly for the handles. Results for the latter, however, could not be corrected for anisotropy, so an unambiguous identification of a shape effect was impossible. In any case it seems that the shape correction is less than other sources of error for samples from the side and base, and all samples used came from these locations.

The anisotropy ratio obtained from these measurements on the skyphos was used to correct the values obtained from the rest of the material. This correction amounted to between 3 and 7% (obviously it could not be larger than 10%). The corrected values for the ancient field obtained from each sherd are plotted

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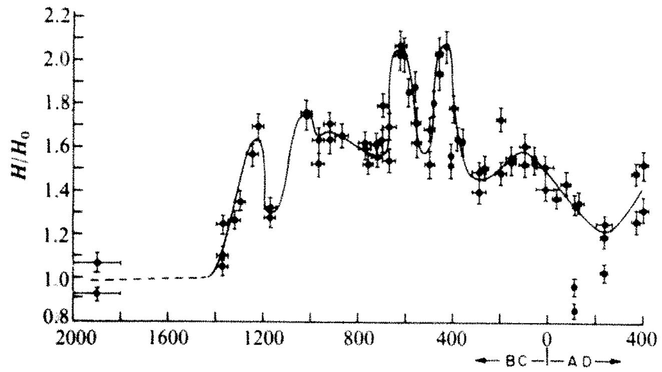

Fig. 1 The change in the ratio of the ancient intensity of the geomagnetic field $\mathbf{H}$, to the present day value, $\mathbf{H}_0$ in Athens. With the exception of the results on the skyphos each point represents a single measurement made on a core taken from a different sherd.

in Fig. 1. These results were obtained on about 80 sherds, and one whole vessel. Nine results were discarded because they displayed evidence of reheating or because alteration occurred before demagnetisation to a useful degree.

The horizontal error bars represent the archaeologists's estimate of the error in the date for the sherd in question. The vertical error bars were determined from the limits on the scatter of the results from the Ashmolean skyphos, namely ±4%. This represents quite an arbitrary estimate of the error involved in obtaining the ancient field from a fragment of pottery. The error may, in fact, be larger than this. For one thing the degree of anisotropy is not necessarily the same. Nevertheless, with few exceptions the error bars for different sherds from the same time period overlap.

The exceptions fall into two categories. First, those sherds for which the field intensity was changing rapidly. In this case a difference in time of manufacture could lead to a difference in field >8%. Second, those sherds which did not, in fact, belong to the time period assigned, but were accidentally included. This is always a possibility with deposits.

Although anisotropy is potentially a large source of error, the 4% uncertainty in our results apparently mainly arises for another reason. This is clear from the scatter in the results obtained on the four cores from the base of the skyphos. The anisotropy correction for all of these must have been very nearly the same, but they still scatter by ~±3%. In an earlier experiment[2] four slices from the same core yielded a scatter of ±3.5%. The reason for the scatter seems to lie in nonlinearities in the plots of $\mathbf{M}_z^H$ against $\mathbf{M}_y^H$ or $\mathbf{M}_z^0$. These nonlinearities occur at both low and high temperatures. The high temperature deviations are undoubtedly caused by physical or chemical alteration of the magnetic minerals. The low temperature difficulties seem to be connected with the cooling rate. At low temperatures, below ~250 °C, it seems that the relaxation times involved are long enough for the moment produced to be significantly affected by the time at temperature, and the rate at which the sample is cooled.

Finally, another difficulty should be mentioned, there was clear evidence that some of the material had been reheated to a high temperature after the initial firing. This was particularly evident in the pottery produced after AD 200. Reheating may be responsible for the increased variability in the field values which is apparent in the results for this more recent material.

The question now arises as to whether the results display the actual change in the geomagnetic intensity or just some systematic variation in the fabric. If the former is true a similar change should be apparent in other locations. Thus, it is interesting to compare these data with those obtained by Bucha[3] in Czechoslovakia. Although the details are not reproduced, the broad features of the two curves agree. In fact, Bucha's material would probably not be sufficient to show both peaks at 600 BC and 450 BC. Bucha[8] also points out that because rotation of the core leads to a westward drift of roughly 0.2° yr^{-1}; the maximum should occur at about 0 BC in America and AD 500 in Japan. Bucha[8] suggests that this is, in fact, apparent in measurements by Bucha *et al.*[4] in Mexico and the southern US, and data obtained by Nagata *et al.*[5] and Sasajima[6] in Japan.

A change in the geomagnetic intensity with time such as that shown in Fig. 1 should be useful as an adjunct to dating. It is tempting to conclude on the basis of the data from Europe, America, and Japan that the broad features of the time dependence of the field intensity at other sites may be predictable, using these data and the rate of westward drift. However, such a conclusion would be premature as this field dependence on time does not occur in all results obtained by others. Nor, if it is present, does it always occur in a time period consistent with a rate of westward drift of 0.2° yr^{-1} (see ref. 7).

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Magnetic anisotropy in ancient pottery

WHILST measuring the thermoremanent magnetism (TRM) of ancient pottery to determine the field magnitude at the time of firing, by using a modification of the Thellier thermal remagnetisation technique[1], we have observed that the ease with which TRM is acquired is dependent on the angle at which the remagnetising field is applied. As a general rule it seems that the hardest direction is perpendicular to the plane of the pottery and that to a rough approximation there is an 'easy plane' lying in the plane of the pottery. The effect is apparently distinct from that due to the demagnetising field associated with sample shape, because the anisotropy effect is observed for sample shapes for which there would be no variation of demagnetising field with change of angle of applied field (such as a cylindrical sample being magnetised at various azimuthal angles in the plane perpendicular to the axis of the cylinder), as well as for samples for which the specific magnetisation is too weak for demagnetising fields to be significant. The anisotropy effect has been observed also in alternating field (750 Hz) measurement of instantaneous susceptibility.

Consideration of demagnetising fields suggests that the TRM acquired by a disk-shaped sample will be greater when the disk is orientated so that its plane is parallel to the field than when it is orientated perpendicular to the field. As reported previously[2], such a difference was observed for disks (diameter 25 mm, thickness 7 mm) of baked clay from a suite of samples for which the specific magnetisation ranged from 4×10^{-3} to $0.06\times$

Table 1 Anisotropy data for various samples of ancient pottery

	Specific magnetisation ($\mathrm{A\,m^2\,kg^{-1}} \times 10^3$)	Ratio of maximum to minimum TRM susceptibility
Romano-British coarse pottery		
(sample 559 a 1.2)	1.1	1.2
Gallo-Roman pottery (Samian ware)		
(561 a 2.1)	0.4	1.6
(1604 a 1.2)	0.3	1.7
(1604 a 4E)	0.3	1.7
Roman tile		
(1631 a)	2.2	1.2
(1631 92)	2.1	1.4
Minoan pottery (LMIA)		
(1635 a 1)	2.5	1.6
Recently fired 'ancient' pottery		
(1607 d.1.1)	3.3	1.7
(1614 c. 3.2)	3.6	1.1
Recent brick		
(IB4)	1.5	1.04

Note that the specific magnetisations quoted are for an applied field of 100 μT in the direction of minimum TRM susceptibility.

$10^{-3}\ \mathrm{A\,m^2\,kg^{-1}}$ (the numerical values are the same when specific magnetisation is expressed in $\mathrm{G\,cm^3\,g^{-1}}$). Consideration of shape demagnetisation effects alone suggested that for the weakly magnetised samples the difference would be negligible; in fact, some of the weakly magnetised samples showed a difference which was comparable with, and in one case greater than, the 30% difference shown by samples at the upper end of the specific magnetisation range. The explanation for this unexpected result may lie in the intrinsic magnetic anisotropy of the baked clay fabric.

The intrinsic anisotropy was first directly observed in squat cylindrical samples (3 mm × 3 mm) cored from Gallo-Roman pottery (Samian ware), using the SQUID cryogenic magnetometer of Walton[3]. In the modified[4] version, the oven is mounted directly below the measuring cavity and the sample, mounted with fireclay on a vertical quartz rod, can be positioned either in the oven or in the cavity. The vertical component of its magnetisation is determined from the SQUID signal resulting from the flux change in the vertical pick-up coils during movement into (or out of) the cavity. The horizontal component is determined from the amplitude of the sinusoidal variation of flux picked up by the horizontal detection coils due to rotation of the sample about a vertical axis at around 2 revolutions per second; the vertical and horizontal detection coils are connected in series to the SQUID element itself. In the oven position, controlled vertical and horizontal steady fields can be applied to the sample; in their absence the residual Earth's field is <0.5 μT because of mu-metal screening. Applied fields in the range 10–100 μT were used in the present work.

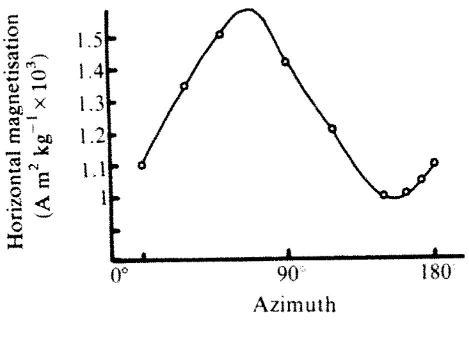

Fig. 1 Variation of TRM with direction of application of the applied field for a sample of Minoan pottery (LM 1A, sample no. 1635 a 1). The vertical scale shows the horizontal component of the TRM resulting from application of a horizontal field of 50 μT; the field was applied while the sample cooled from 500 to 150°C. The sample was a 3 mm × 3 mm cylinder cored with its axis perpendicular to the plane of the pottery. It was mounted for measurment with its axis horizontal and at zero azimuth on the scale shown; the orientation was only approximate and it is considered that the maximum is displaced from 90° because of this.

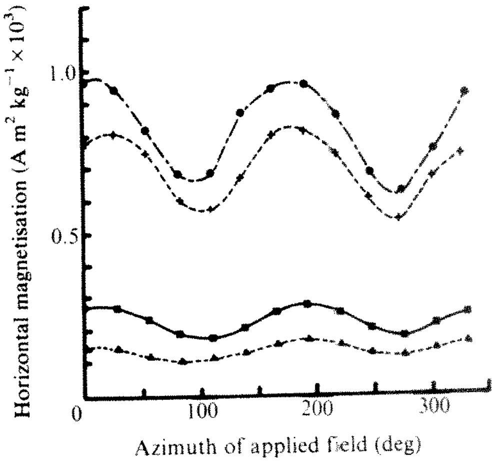

Fig. 2 Variation of TRM with direction of applied field for a sample cooled in a horizontal field of 50 μT over various temperature ranges. ●, 450–300°C; +, 300–150°C; ■, 150–20°C; ▲, 600–450°C. The data points through which the full line is drawn were obtained by cooling the samples from 150° to 20°C in the presence of the applied field. Similar curves were produced for different temperature ranges by cooling in the field from the higher temperature stated switching off the field at the lower temperature. The sample was a 3 × 3 mm cylinder mounted with its axis approximately vertical. It had been obtained by coring parallel to the plane of a piece of pottery (1607 d 1) that had been fired in AD 1962 in a replica of a Romano-British kiln at Boston, Lincs.

In Samian ware samples, for a sample cored with its cylindrical axis perpendicular to the plane of the pottery the TRM resulting from a field along the axis was 30–40% lower than the TRM resulting from the same field applied perpendicular to the axis. Conversely for a sample cored with its axis parallel to the plane of the pottery the TRM resulting from a field along the axis was 30–40% higher than for the field applied perpendicular to the axis. Table 1 lists results for this and other samples and Fig. 1 shows the variation of resultant TRM with angle of applied field for one of them. It has also been observed, for two samples at any rate, that the degree of anisotropy is the same for partial TRM's given over different temperature ranges; this is shown in Fig. 2.

A consequence of fabric anisotropy is that an applied field gives rise to a small TRM component transverse to the direction of the field unless the field is either exactly parallel or perpendicular to the 'easy plane'; if the applied field makes an angle α with the easy plane then the ratio of the transverse TRM component to the parallel component is given by

$$\frac{(a-1)\tan\alpha}{a+\tan^2\alpha}$$

where a is the ratio of the TRM susceptibility within the easy plane to the susceptibility perpendicular to that plane, the susceptibility within the easy plane being taken as uniform. This transverse component has been observed and in Fig. 3 we show the vertical TRM component resulting from application of a horizontal field at various azimuths, zero azimuth being taken as the direction of the horizontal component resulting from an applied vertical field. The maximum of the vertical component occurs at this zero azimuth as expected from consideration of the easy plane model. In contrast, the azimuthal variation of the horizontal component (resulting from the applied horizontal field) is not as predicted from the model; this is because it is masked by variation of susceptibility within the easy plane. As mounted for the measurements of Fig. 3 the ratio of the average horizontal TRM susceptibility to the vertical susceptibility was

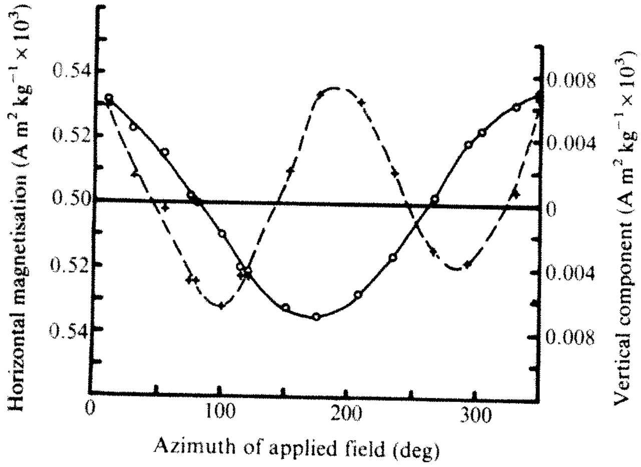

Fig. 3 Horizontal (+) and vertical (O) components of the TRM resulting from horizontal applied field of 100 μT applied at the azimuth shown, zero azimuth being taken as the direction of the horizontal component of the TRM that results from a vertical field; the field was applied while the sample cooled from 500° to 150°C. The sample was a 3×3 mm cylinder obtained from a piece of Samian ware (561 a 2.1) by coring perpendicularly to the plane of the pottery; it was mounted for measurement with its axis roughly vertical.

1.43 and application of a vertical field produced a horizontal TRM component which was 21% of the vertical component; these data indicate that the easy plane was inclined at an angle of ~25° to the horizontal and that the ratio of the average TRM susceptibility within the easy plane to the susceptibility perpendicular to it is 1.6.

The explanation that we propose for this intrinsic anisotropy in the fabric of pottery is that it is due to preferential alignment of magnetic grains. The alignment presumably occurs during the moulding of the clay into shape and that perhaps it is further enhanced by grain growth during firing. Whatever its cause the effect dictates that for reliable determination of ancient field by thermal remagnetisation it is important to apply the laboratory field in such a direction as to produce a TRM in the same direction as the magnetisation that was acquired in antiquity, or to make appropriate correction. Failure to do either could lead to an error of up to 60% in the case of the sample on which Fig. 1 is based, and to ensure that the error from misalignment is kept below 5% it is necessary to have the direction of the laboratory TRM within 10° of the direction of the ancient magnetisation, as will be seen from Fig. 1. Note that in the classic work of Thellier and Thellier[1] the samples concerned were from bricks, tiles and kilns; at any rate for the two former the data of Table 1 suggest that the effect may be relatively unimportant. The pottery listed in Table 1 is presumed to have been made on a wheel; however, the alternating field measurements included some non-wheel-made pottery of the British Iron Age and this too showed some effect. This and other aspects are being investigated further.

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Correlation between heart attacks and magnetic activity

SINCE the pioneering work of Düll and Düll[1], many papers have been published on the possible correlation between geomagnetic activity and medical phenomena. Most of them conclude that there is such a correlation, although this may reflect the tendency to publish positive results and discard negative ones, and there is widespread scepticism concerning the reality of the correlation. The main objections concern the validity of the statistical tests, failure to remove trend and inadequate allowance for seasonal variations. Here we present data for which the correlation is particularly high, and can be demonstrated convincingly by standard statistical tests.

The medical data consist of the number of cardiac emergencies admitted each day to the cardiac/thoracic wards of the main hospitals in Hyderabad and Secunderabad, the Osmania General Hospital and the Gandhi Hospital, respectively, during 1967–1972. Almost all the acute heart cases in those cities are admitted to the two hospitals except a few that are treated privately. The geomagnetic data consist of daily sums of Kp, the planetary index of geomagnetic activity for 3-hourly intervals of UT. The Kp index is intended to measure the geomagnetic effect of solar particle flux. Values of Kp are published, together with details of their definition and derivation, by the International Union of Geodesy and Geophysics[2]. The daily sums of Kp cover an interval of 24 h starting from 18 h UT, whereas Indian Time starts at 18.5 h UT, so there is a small lag of 30 min between the geomagnetic and medical data. The interval 1967 to 1972 was selected before the data were examined.

The geomagnetic data show marked variations with season, and similar variations are seen in the medical data (Fig. 1). This is to be expected if they are correlated, but the seasonal variations in the medical data might equally be due to other (for example, meteorological or dietary) causes. To eliminate such seasonal effects, and also to avoid long-term trends (which are in any case small, Table 1), we have considered the data a month at a time.

Table 1 Annual mean values

Year	Kp sum	Daily admissions
1967	15.9	2.35
1968	18.4	2.72
1969	15.9	2.51
1970	16.1	2.10
1971	16.2	2.25
1972	16.8	2.49

Table 2 lists, for each month, the coefficient of correlation, r, between the medical, M, and geomagnetic, G, parameters, and also the constants a and b for the relation $G = aM + b$, obtained by the method of least squares. The results for the first few months are presented graphically in Fig. 2; as the correlation coefficients confirm, the remaining months are essentially similar. In each case, there is a clear tendency for the number of admissions to increase with magnetic activity.

To estimate the probability of such correlations occurring by chance, Student's t test would seem appropriate, but for this test to be valid, the data need to be approximately representative of a normal distribution, and both the G and M data are heavily biased towards the lower end of their range. The standard way of making such distributions more nearly normal is to take logarithms, and this is quite satisfactory for G, but for M (which includes zero values) a constant must first be added. We have chosen the constant to be 10, as this value approximately equalises the positive and negative moments about the mean.

Table 2 Data for each month

Month 1967	r	a	b	r'	P
Jan	0.77	3.6	5.5	0.71	2.1×10^{-3}
Feb.	0.80	3.8	5.1	0.79	7.7×10^{-4}
Mar.	0.68	3.1	5.7	0.60	1.4×10^{-2}
Apr.	0.57	2.6	9.2	0.56	3.0×10^{-2}
May	0.76	4.0	7.5	0.70	2.5×10^{-3}
June	0.61	2.0	10.9	0.62	1.4×10^{-2}
July	0.73	2.9	8.4	0.68	3.8×10^{-3}
Aug.	0.56	2.5	11.5	0.50	4.9×10^{-2}
Sep.	0.83	4.7	7.8	0.78	6.0×10^{-4}
Oct.	0.74	3.8	5.6	0.73	1.3×10^{-3}
Nov.	0.56	4.1	7.8	0.48	7.0×10^{-2}
Dec.	0.73	3.8	7.7	0.71	2.1×10^{-3}
1968					
Jan.	0.63	2.2	12.4	0.58	1.9×10^{-2}
Feb.	0.78	4.5	6.0	0.78	6.0×10^{-4}
Mar.	0.56	2.1	13.7	0.51	4.4×10^{-2}
Apr.	0.51	3.1	11.4	0.37	1.7×10^{-1}
May	0.74	3.2	11.5	0.68	3.8×10^{-3}
June	0.60	4.3	10.0	0.53	4.2×10^{-2}
July	0.63	2.8	11.6	0.57	2.1×10^{-2}
Aug.	0.60	2.7	9.7	0.55	2.7×10^{-2}
Sep.	0.81	3.9	7.8	0.80	3.4×10^{-4}
Oct.	0.77	4.2	4.8	0.72	1.7×10^{-3}
Nov.	0.77	5.2	1.1	0.67	6.3×10^{-3}
Dec.	0.68	2.3	8.9	0.69	3.1×10^{-3}
Month 1969	r	a	b	r'	P
Jan.	0.56	2.6	7.8	0.60	1.4×10^{-2}
Feb.	0.39	2.1	11.7	0.32	2.6×10^{-1}
Mar.*	0.73	3.7	7.2	0.63	1.2×10^{-2}
Apr.*	0.36	1.4	16.5	0.30	3.0×10^{-1}
May	0.76	4.6	6.8	0.67	4.5×10^{-3}
June	0.51	3.0	10.7	0.58	2.3×10^{-2}
July	0.70	3.6	5.8	0.71	2.1×10^{-3}
Aug.	0.40	1.7	9.9	0.38	1.5×10^{-1}
Sep.	0.81	3.9	5.5	0.73	2.0×10^{-3}
Oct.	0.72	4.2	4.6	0.72	1.7×10^{-3}
Nov.	0.78	4.0	4.4	0.69	4.4×10^{-3}
Dec.	0.53	2.9	5.6	0.52	3.9×10^{-2}
1970					
Jan.	0.41	1.3	10.3	0.31	2.4×10^{-1}
Feb.	0.50	2.6	6.8	0.56	3.7×10^{-2}
Mar.	0.87	4.1	6.4	0.81	1.4×10^{-4}
Apr.	0.62	4.3	9.7	0.65	8.7×10^{-3}
May	0.55	2.4	11.0	0.51	4.4×10^{-2}
June	0.85	5.1	7.5	0.79	4.6×10^{-4}
July	0.85	3.7	11.3	0.77	4.8×10^{-4}
Aug.	0.82	4.4	7.5	0.77	4.8×10^{-4}
Sep.	0.69	3.6	10.0	0.58	2.3×10^{-2}
Oct.	0.65	3.3	9.4	0.55	2.7×10^{-2}
Nov.	0.73	3.0	9.8	0.62	1.4×10^{-2}
Dec.	0.77	3.1	6.8	0.69	3.1×10^{-3}
Month 1971	r	a	b	r'	P
Jan.	0.79	3.8	6.7	0.61	1.2×10^{-2}
Feb.	0.66	3.1	10.3	0.55	4.2×10^{-2}
Mar.	0.74	3.8	6.9	0.72	1.7×10^{-3}
Apr.	0.64	2.6	12.1	0.62	1.4×10^{-2}
May	0.90	4.3	8.4	0.81	1.4×10^{-4}
June	0.76	4.6	8.8	0.69	4.4×10^{-3}
July	0.41	1.5	10.8	0.43	9.6×10^{-2}
Aug.	0.49	2.8	9.7	0.45	8.0×10^{-2}
Sep.	0.71	3.2	9.2	0.73	2.0×10^{-3}
Oct.	0.78	4.4	7.6	0.81	1.4×10^{-4}
Nov.	0.83	4.3	3.9	0.68	5.3×10^{-3}
Dec.	0.80	4.4	3.7	0.69	3.1×10^{-3}
1972					
Jan.	0.65	2.9	10.6	0.51	4.4×10^{-2}
Feb.	0.55	1.9	10.9	0.48	7.0×10^{-2}
Mar.	0.70	3.4	8.4	0.63	8.9×10^{-3}
Apr.	0.71	3.1	9.3	0.61	1.6×10^{-2}
May	0.72	2.7	10.2	0.70	2.5×10^{-3}
June	0.79	4.0	7.7	0.71	3.0×10^{-3}
July	0.32	1.6	9.6	0.23	3.9×10^{-1}
Aug.	0.85	4.7	7.5	0.77	4.8×10^{-4}
Sep.	0.63	3.3	8.4	0.65	8.7×10^{-3}
Oct.	0.64	3.8	7.7	0.68	3.8×10^{-3}
Nov.	0.77	4.0	7.0	0.71	3.0×10^{-3}
Dec.	0.61	3.2	6.4	0.58	1.9×10^{-2}

* Medical data are not available for 9 and 10 March, 11 and 12 April 1969.

The coefficient of correlation, r', between log G and log $(M+10)$ is given in the penultimate column of Table 2. In general, r' is similar to but slightly less than r. The final column of Table 2 lists the probability of such a correlation occurring by chance, according to Student's t test, using $t = r'/[(1-r'^2)/\nu]^{1/2}$, where ν denotes the number of degrees of freedom in the data for 1 month[3]. If the data for successive days were completely independent, ν would be $N-2$, where N is the number of days in the month. However, it is certainly true in the case of magnetic activity, and may also be true for heart attacks, that the data show considerable conservation; that is, a high value is likely to be followed by another high value; and this may considerably reduce the effective number of degrees of freedom[4]. Following Taubenheim[5], we find that, for our data, the effective number of degrees of freedom is $\nu = N/2.3-2$ for log G and $\nu = N/1.9-2$ for log $(M+10)$, and the second value of ν is that appropriate for significance tests. The values of P in Table 2 were deduced using the integral part of ν from the second relation.

From uncorrelated data we might expect 3 or 4 of the 72 correlation coefficients to achieve the 5% level of significance ($P < 5\times10^{-2}$), but we find that all except 10 are significant at this level. Similarly, we find 38 to be significant at the 1% level, and 11 at the 0.1% level, compared with 1 and 0 to be expected by chance.

Because there is no *a priori* reason to expect any conservation in the medical data, the presence of such conservation at a level similar to that in the geomagnetic data provides some additional support for the hypothesis that there is an association between the two phenomena. Also it should be noted that, although the adjustments for lack of normality and for conservation seem to complicate the analysis, had either of these been omitted, the level of significance would have appeared to be even higher.

Why should these data show such a high correlation, when those of Lipa *et al.*[6] for the times of deaths in the United States due to coronary heart disease and stroke during 1962–1966 showed no significant correlation with magnetic activity? First, the level of artificial magnetic disturbance (from cars, industry, domestic appliances and so on) is very much lower in India than in the United States. Second, they considered the time of death, which may occur much later than the medical circumstance which was its cause.

It may be objected that our data and method of analysis are not the most appropriate. Some of the admissions of cardiac emergencies may have occurred a day or more after the onset of the condition, others may have been delayed for administrative reasons (for this reason, days of zero admissions were omitted from a previous study[7]). A proportion of the cardiac emergencies may have been diagnosed wrongly. Perhaps we should have used a local measure of magnetic activity, as was done by Srivastava *et al.*[7], but, because one of us is responsible for the measurement of these, it was thought best to use an international parameter to avoid any suspicion of bias. (In any case, the difference between Kp and the local measure of K is always small.) Maybe a better result would have been obtained if we had introduced an appropriate time-lag between the magnetic and medical parameters. However, none of these possibilities would be expected to decrease the correlations, and we have analysed our data in the most straightforward way possible in the hope of convincing the reader that, in this case at least, there is a significant correlation between medical and geomagnetic phenomena.

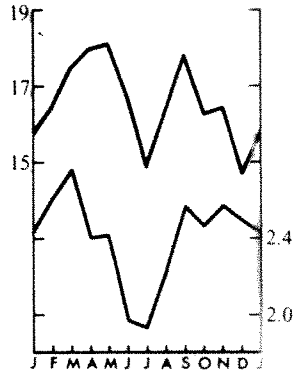

Fig. 1 Monthly mean values of the daily sums of magnetic activity indices, Kp (upper graph) and the daily admissions of cardiac emergency cases (lower graph).

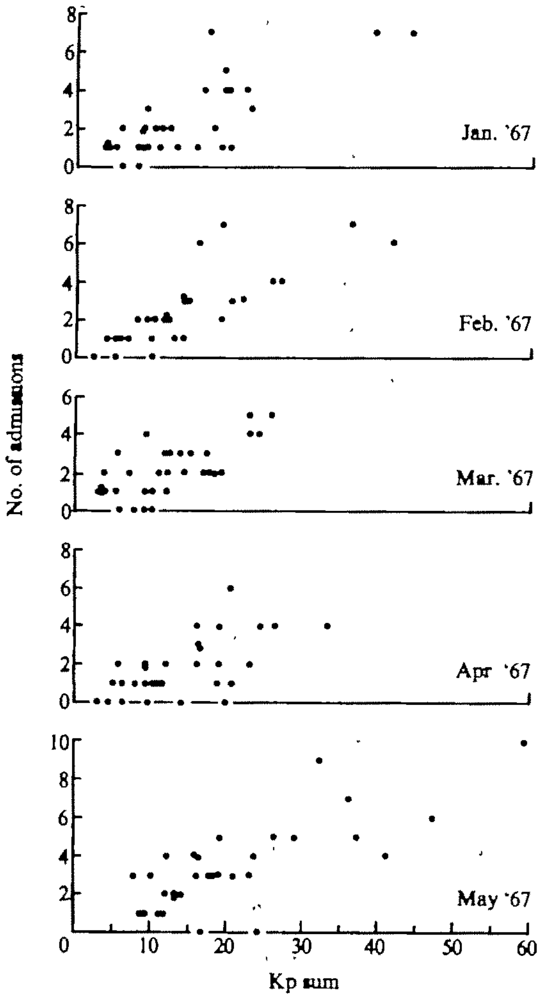

Fig. 2 Plots of daily admissions of cardiac emergency cases against daily sums of magnetic activity indices, Kp, for the first 5 months of data.

The possibility that there is some other cause (of solar origin?) responsible for both the magnetic and medical phenomena should not be ignored.

We thank the Superintendents of the Osmania General Hospital, Hyderabad, and the Gandhi Hospital, Secunderbad, for making available their hospital records, and our colleagues at the Hyderabad Magnetic Observatory for abstracting the medical data from these records.

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Evidence that geomagnetic variations can be detected by lorenzinian ampullae

THERE have been several reports of electroreceptor responses in elasmobranchs to an electric field induced by various magnetic stimuli (including water flow in a geomagnetic field)[1–6]. The possibility was suggested that fish can detect telluric currents produced by geomagnetic variations[7], but it was not investigated experimentally. Here we describe direct neurophysiological evidence for such a phenomenon.

Experiments were carried out on Barents Sea skates *Raja radiata* in Dalnje-Zelenetsk Bay, on the Barents Sea. Single-unit activity was recorded as previously described[5] from nerve fibres supplying the ampulle of Lorenzini of the hyoid ampullary group. Impulses were processed by an integrator so as to follow the spike frequency in the course of the experiment.

The electrical state of the sea was monitored using two Ag–AgCl electrodes 400 m apart, parallel with the coastline. The two electrodes were connected by a marine cable to similar electrodes in the experimental tank. The electrodes in the tank were 1 m apart. To equalise the voltage gradients in the sea and the tank, two resistors (R_1 and R_2) were interposed, as shown in Fig. 1. The resistors were chosen by the following reasoning. Voltage gradient in the tank is given by

$$E_t = E_s \frac{l_s R_t}{l_t(R_l + R_1 + R_2 + 2R_e + R_t)}$$

where E_s is voltage gradient in the sea; l_s is the distance between marine electrodes, 400 m; l_t is the distance between the tank electrodes, 1 m; R_t is water resistance in the tank, 45 Ω; R_l is the overall resistance of electric line (seawater resistance + marine electrodes resistances + cable resistances), 100 Ω; R_e is the

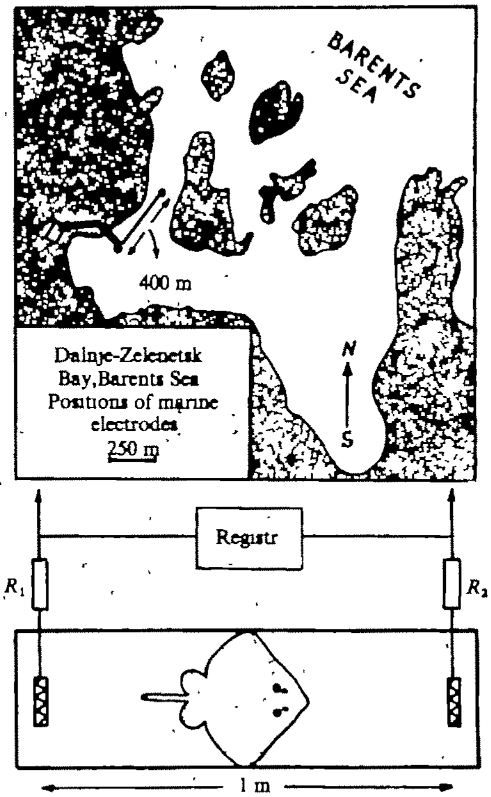

BARENTS SEA
400 m
Dalnje-Zelenetsk Bay, Barents Sea Positions of marine electrodes
250 m
N
S
Registr
R_1
R_2
1 m

Fig. 1 Experimental set-up For details see the text.

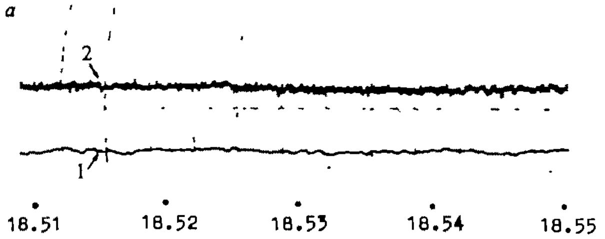
resistance of an electrode in the tank, 30 Ω; $R_1 = R_2$ are resistances in series.

To make E_t equal to E_w, R_1 and R_2 should be

$$R_1 = R_2 = 1/2[(l_w R_t/l_t) - R_1 - 2R_e - R_t] \approx 9 \quad \text{k}\Omega$$

To estimate the electroreceptor unit response to natural stimulus, spike frequency was compared to the magnitude of the voltage gradients produced by telluric currents. Magnetic field variations were registered.

Measurements were made during a magnetic substorm which appeared together with the polar light on 25–26 February, 1978. The rate of change in the geomagnetic vertical component at the time when measurements were made was 0.2 to 1.0 nT s^{-1} (2×10^{-6}–10^{-5} G s^{-1}); maximal deviation from the background field was 216 nT. Short-period (1–3 min) oscillations of telluric voltage gradients were observed to be synchronous with variations of the geomagnetic field. Voltage gradients in the sea (and, consequently, in the tank) were from ±0.6 μV cm^{-1} to (at times) ± 1.25 μV cm^{-1}.



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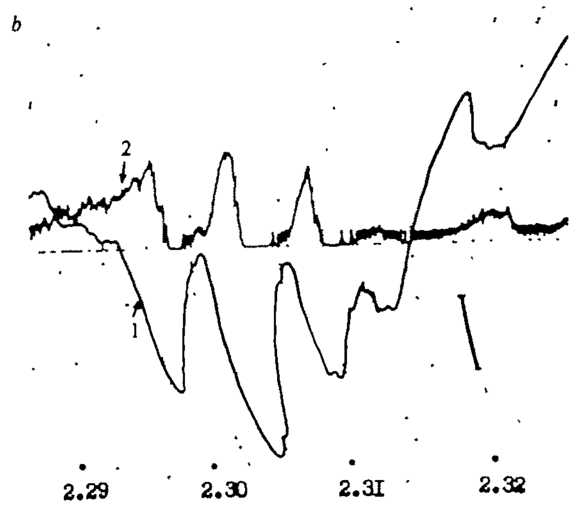

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Fig. 2 Synchronous recording of induced electric field in the sea (1) and the time course of spike frequency of a single electroreceptor (2) before (*a*) and during the magnetic substorm (*b*). Vertical bar: 0.2 μV cm^{-1}, 15 imp s^{-1} (GMT).

Figure 2 shows that electric field oscillations due to geomagnetic variations produced a clear-cut response in the electroreceptors, as judged by corresponding changes in spike frequency. The spike frequency was sensitive not so much to the absolute value of the electric field as to its rate of change, that is, the field derivative with respect to time. Such a response may be due to adaptation of the receptor to a prolonged stimulus. Receptor sensitivity was sufficient to detect a change in the electric field as small as 0.006 μV cm^{-1} s^{-1}.

These measurements clearly indicate that the ampullae of Lorenzini can detect the electric field induced by variations of the Earth's magnetic field. This capacity is likely to be more strongly expressed within the coastal area, and this could be the result of an enhancement of telluric currents near the coast due to 'coast effect' (ref. 8). Such a suggestion agrees with experimental data indicating that the electroreceptor response associated with changes in magnetic field is maximal when a fish is near the wall of a tank[6]. The marine electrodes in our experiments were placed in a bay screened from the open sea by islands which reduced the coast effect of telluric currents[9]. Otherwise, the effects observed might have been greater.

Further studies should provide information about the biological role and practical applications of the detection of telluric currents by fish.

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Correlation between heterozygosity and subunit molecular weight

GENIC heterozygosity at enzyme loci has been examined for correlation with parameters as diverse as substrate specificity[1], physiological function[2] and quaternary structure[3]. However, none of these has provided an adequate explanation for allozymic variation in general. One report[4] suggested a positive relationship for *Drosophila* enzymes between heterozygosity and subunit molecular weight (MW), which can be equated to the size of the structural gene element. Various models, both neutral and selective, would predict a correlation between gene size and variability[5–7]. Recently, a similar correlation has been found within three classes of vertebrates[8,11], and between the number of rare alleles and subunit size in human populations[9,10]. We report here that we have tested the relationship between heterozygosity and subunit size in *Drosophila* species with a new selection of enzymes. Our results indicate that the correlation is indeed general. The relationship is quasi-linear and does not differ significantly from several models. The results support the hypothesis that there are constraints on the number of sites available to charge substitution within an enzyme molecule.

For our test we used data on six of the enzymes used by Koehn and Eanes[4] and estimated the MWs on a series of at least seven enzymes in *Drosophila melanogaster* for which such information was not previously available. We excluded several enzymes from our analysis because of inadequacies in the data. No esterases or phosphatases were used as their homology across *Drosophila*

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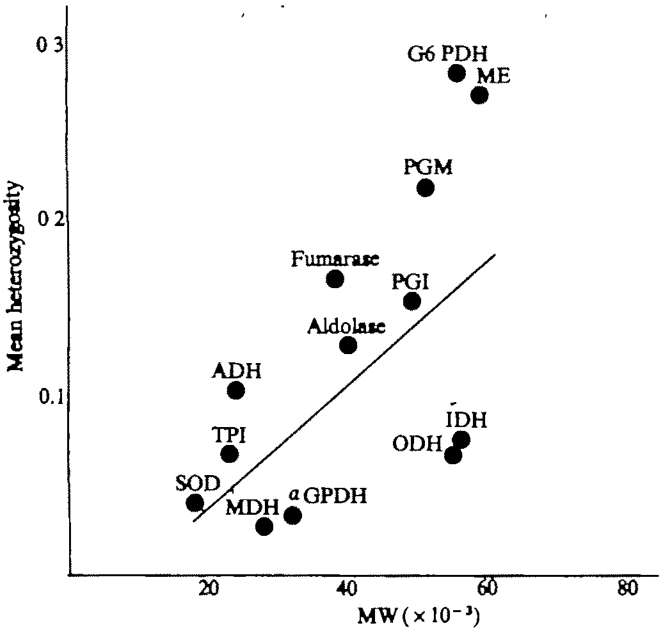

Fig. 1 Mean heterozygosity as a function of MW. SOD, 18,000; TPI, 23,000; ADH, 24,000; MDH, 28,000; αGPDH, 32,000; fumarase, 38,000; aldolase, 40,000; PGI, 49,000; PGM, 51,000, ODH, 55,000; G6PDH, 55,000; IDH, 56,000; ME, 58,000. Each point represents the mean heterozygosity value for an enzyme over all species surveyed for that enzyme. The regression line indicated ($y = 0.00000353x - 0.0289$) was calculated from the full data set of 118 observations (which is available on request).

species is not clear. In addition to these restrictions on the enzymes used we have made a new selection of population data. In so doing, we only used data from those sources which permitted calculation of the actual numbers of each allele observed. However, whereas Koehn and Eanes[4] did not consider samples where an enzyme was completely monomorphic, we included all such samples.

The sedimentation constants ($s_{20,w}$) of phosphoglucose isomerase (PGI) and triosephosphate isomerase (TPI) were estimated from sucrose density gradients[11], relative to *D. melanogaster* alcohol dehydrogenase (ADH) and rabbit muscle lactate dehydrogenase (LDH). The means and standard errors of six estimates were: PGI, 6.6±0.23; TPI, 4.5±0.13. Stokes' radius estimates of 35.5 Å and 28.6 Å, respectively, were obtained from gel filtration relative to three standards[12]. From these data, the native MWs of these enzymes were calculated to be 97,000 for PGI, and 47,000 for TPI, assuming a partial specific volume of 0.725 $cm^3 g^{-1}$.

The MWs of enzymes with too little activity for convenient assay in the sucrose density gradients were estimated directly from gel filtration chromatography[13]. Relative to five standards, the native MWs of fumarase, isocitrate dehydrogenase (IDH) and phosphoglucomutase (PGM) were estimated as 150,000, 112,000 and 51,000, respectively. Full data on the MW determinations of these and other *Drosophila* enzymes will be published elsewhere.

The enzymes used in investigating the relationship between heterozygosity and subunit size were: TPI, PGI, IDH, fumarase and PGM from above; malic enzyme (ME) and glucose 6-phosphate dehydrogenase (G6PDH) for which direct estimations of subunit size (58,000 and 55,000) have been made in our laboratory from purified enzyme[14], and, from Koehn and Eanes' analysis[4], superoxide dismutase (SOD), malate dehydrogenase (MDH), α-glycerophosphate dehydrogenase (αGPDH), octanol dehydrogenase (ODH), aldolase and ADH. The structures of the new enzymes have been established mainly by starch-gel electrophoresis. Other work in progress in our laboratory has necessitated the collection of variants at all these loci in *D. melanogaster*. From this work, TPI, PGI and IDH have been deduced to be dimers from the appearance of three clear bands in heterozygotes, whereas PGM is a monomer and fumarase and aldolase are tetramers (R. A. Voelker and C.H.L., unpublished data). For the remaining enzymes, the structure given by Koehn and Eanes[4] was assumed.

The population data used in the analysis were carefully selected, as has been described. (They were taken from refs 15–25 and from unpublished data of F. J. Ayala.) Where more than one suitable sample was available for a species, the data were pooled so that each species was given equal weight. Data from a total of 15 species were used in the analysis. The correlation analysis was carried out on the new enzyme set, on the set used by Koehn and Eanes, and on both combined. A non-parametric test for rank correlation was made using Kendall's τ, as neither variable is normally distributed, and the results are given in Table 1. It can be seen that there is a highly significant correlation between heterozygosity and subunit MW in the combined data set. Figure 1 shows the distribution of the data and a least-squares regression line which was fitted to the whole data set. For clarity, only one point is shown for each enzyme, the mean value taken over all species with data for that enzyme.

We have been able to confirm the conclusion of Koehn and Eanes[4] that there is a positive correlation between allozymic heterozygosity and subunit MW. Having made a stringent selection of the data and used a test of reduced power we are satisfied that this relationship is general. Nei *et al.*[10] have recently examined the relationship between MW and heterozygosity in vertebrates and obtained a significant correlation on data from three classes. These authors claimed that the magnitude of their correlation was "roughly" in agreement with the predictions of a neutral model. We have examined our data in a similar way and have also found agreement between our correlation and the predictions of both the infinite alleles[5] and the charge-state models[26]. However, the regression line has a wide confidence belt and almost any model which predicts a positive monotonic relationship between the two variables would also fit our data. Two alternative such models are those of Gillespie[6] and Watterson[7]. In Gillespie's random-environments model the heterozygosity at a locus depends inversely on the relative effect of a substitution on enzyme function. In general, the larger an enzyme molecule, the smaller would be the relative effect of a mutation. In Watterson's stochastic heterotic model heterozygosity is as predicted by the infinite-alleles model when $\sigma = 2N_e s = 0$. We found that although the predictions diverge as σ increases, for $\sigma = 0(1)$ this model does not differ greatly from the neutral case in the region of heterozygosity values of our data.

We have also carried out a two-way analysis of variance on our heterozygosity data by gene and species, and the results

Table 1 Correlation between allozymic heterozygosity and subunit MW

Data set	Kendall's τ	Probability of a higher τ value
(1) TPI, PGI, IDH, PGM, ME, fumarase, G6PDH	0.159	0.13
(2) ADH, Aldolase, SOD, MDH, ODH, αGPDH	0.102	0.26
(3) All data—(1)+(2)	0.213	0.0013

The MWs of TPI and PGI were determined by sucrose density gradient sedimentation and those of fumarase, IDH and PGM by gel filtration on Sephacryl S-200. The structure of these enzymes was determined by starch-gel electrophoresis as described in the text. Subunit sizes were thereby assigned as follows: TPI, 23,000; PGI, 49,000; fumarase, 38,000; IDH, 56,000; PGM, 51,000. Subunit sizes for ME (58,000) and G6PDH (55,000) have been determined from purified enzyme. Data set (1) consists of those enzymes whose MW was not previously known. Data set (2) consists of enzymes previously used in the analysis of Koehn and Eanes[4]. In each case new population data were selected as indicated in the text. Note that G6PDH was used in Koehn and Eanes' analysis[4]. It is included in data set (1) because a new MW determination is available which represents a substantial change from the value previously used.

Table 2 Analysis of variance of heterozygosity by gene, species and MW

	Sum of squares	Degrees of freedom	Variance component per observation
Species	0.4792*	14	0.0041
Gene	0.6365†	12	0.0055
MW	0.2328		0.0020
Other gene effects	0.4037		0.0035
Error	1.7973	91	0.0153
Total	2.913	117	0.0249

The analysis of variance was first carried out by gene and species on the full data set. The component of the variance due to gene effects was then partitioned into that due to MW and that due to other gene-specific effects as described in the text. The level of significance of the correlation with MW is given in Table 1.
*Significant at 5% level.
†Significant at 1% level.

(Table 2) show significant effects due to both. We have calculated the correlation coefficient between heterozygosity and MW, corrected for species effects ($r = 0.309$; $P < 0.01$). Following Nei *et al.*[10], r^2 is the ratio of the co-variance between gene-average heterozygosity and MW to the product of the variance in MW and the total variance in heterozygosity less that due to species. We were thus able to divide the genic component of the variance into that due to MW and that due to other gene effects. By partitioning the variance in heterozygosity in this way, we have been able to compare the component due to MW with that due to species. We find (Table 2) that the variance component due to MW is about half that due to species.

In the infinite-alleles model of Kimura and Crow[5], heterozygosity is determined by the product of effective population size and mutation rate. Assuming that the latter is proportional to structural gene size, it is interesting that the variance component of our data that is due to MW is so large relative to the species effects, as there is only a threefold range in MW. This would suggest that in equilibrium conditions the range in total population size, being the main species effect under this model, would be of the order of sixfold. We consider this a challenge to the infinite-alleles model. Although one possible interpretation could be that the effective population number of these 15 *Drosophila* species only varies to a similar degree, this is unlikely as the sample includes both equatorial forest species (for example, *D. willistoni*) and cactophilic species (*D. arizonensis*). It could also be argued that they have not attained equilibrium due to fluctuations of population size, but it seems more plausible to us that there are general constraints on the number of sites in a gene where a charge substitution is 'evolutionarily acceptable'. This alternative has been suggested by the analysis of the evolution of amino acid sequence in cytochrome *c*, fibrinopeptide A and haemoglobin[27–29]. Werhahn and Gulizia[30] have pointed out that the effect of population size on equilibrium heterozygosity decreases with the number of mutable sites. Thus, the infinite-alleles model is not consistent with all aspects of our data, and although it could be said to predict a correlation between heterozygosity and MW, so could several other models.

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Acetylcholine-elicited responses in primary and secondary mammalian oocytes disappear after fertilisation

THE recent finding[1] of acetylcholine (ACh) receptors on the amphibian oocyte membrane raises two main questions: (1) is the presence of ACh receptors a feature common to all vertebrates including mammals, and (2) does the sensitivity to ACh undergo developmental changes throughout oocyte growth and maturation, or following fertilisation? We have investigated these problems using conventional electrophysiological techniques, and show here that depolarising and/or hyperpolarising membrane potentials are elicited by ACh in mouse dictyate and metaphase II oocytes, but not after fertilisation.

Fully grown dictyate mouse oocytes from antral follicles (see legend to Fig. 1 for technical details) had a resting membrane potential ranging from −20 to −40 mV(-27.1 ± 1.1, mean ± s.e.m., $n = 57$). When these cells were perfused with a medium containing 1×10^{-4} to 2×10^{-2} M ACh, a dose-dependent membrane depolarisation usually occurred (Figs 1, 2*a*). The ACh-elicited depolarisation took many seconds to develop, as previously shown in *Xenopus* oocytes[1], and the delay between ACh application and the onset of the response was never shorter than 15–20 s. Such a delay was not significantly modified by increasing the bath temperature to 37 °C. At lower ACh concentrations (1×10^{-8}–1×10^{-5} M), the response to ACh differed among the oocytes studied. Some oocytes underwent membrane hyperpolarisation after an initial depolarisation, whereas others displayed only the hyperpolarising response, or no resting potential change at all. The hyperpolarising response was only occasionally observed at high ACh doses (up to 2 mM)(Fig. 2*b*). Both depolarising and hyperpolarising ACh potentials gradually 'desensitised' during maintained application of the drug (Fig. 2*a*, *b*), and preliminary exposure of oocytes to ACh concentrations as low as 10^{-11} M completely prevented all membrane responses to maximal ACh doses (up to 2×10^{-2} M). The ACh-elicited depolarisation was associated with a significant decrease in membrane input resistance (see legend to Fig. 1 for technical details), whereas the hyperpolarisation was accompanied by a significant increase in membrane input resistance. Ionophoretic application of ACh on to oocyte membrane occasionally elicited very small depolarising or hyperpolarising responses in antral oocytes, and here again these responses took many seconds to develop, as previously shown in *Xenopus* oocytes[1].

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Incubation of antral mouse oocytes in Ca^{2+}-free (Ca^{2+} replaced by Mg^{2+}), Na^+-free (Na^+ replaced by choline), or K^+-free (K^+ replaced by Na^+) media did not seem to modify the ACh-elicited depolarisation. However, in the presence of Cl^--free medium (Cl^- replaced by SO_4^{2-} or propionate), the ACh depolarising response was enhanced, although a maintained incubation of the cell in Cl^--free medium completely prevented the depolarising response. These findings apparently indicate that chloride ions are involved in the ACh-elicited depolarisation. Similar conclusions were drawn in *Xenopus* oocytes[1]. Similarity between amphibian and mammalian oocyte ACh receptors is further indicated by the ineffectiveness of (+)tubocurarine (up to 10^{-4} M) in blocking responses in antral mouse oocytes, and by the effectiveness of atropine (10^{-7}–10^{-6} M) in preventing ACh membrane depolarisation. Atropine did not seem to abolish the ACh-elicited hyperpolarisation.

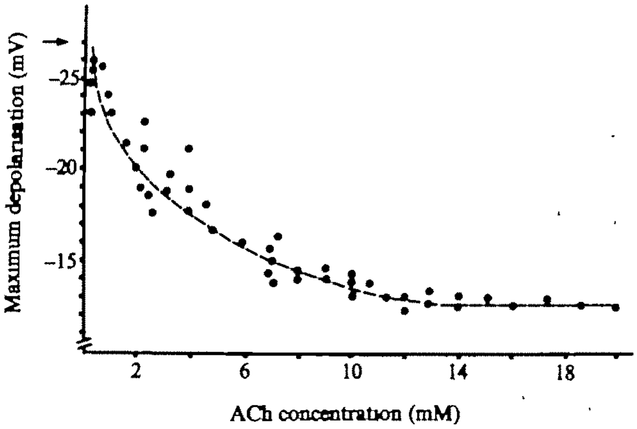

Fig. 1 Depolarising response of antral mouse oocytes in the presence of increasing ACh concentrations. The maximum depolarisation level after ACh perfusion obtained in 47 independent determinations is plotted against the corresponding ACh concentration in the perfusion bath. The arrow indicates the mean membrane resting potential of the tested cells. Swiss CD1-COBS mice from Charles River Italia were used in all experiments. Dictyate oocytes at an intermediate stage of growth (vitellus diameter 60–65 μm, pre-antral, from follicle stages 5a and 5b, ref. 2) and at the end of growth (vitellus diameter 75–85 μm, antral, from antral follicles) were obtained mechanically, by pricking infant (pre-antral) and adult (antral) ovaries with a needle, and were completely freed from surrounding granulosa cells by gentle pipetting in phosphate-buffered saline (PBS) supplemented with pyruvate, lactate and bovine serum albumin. Metaphase II oocytes and fertilised eggs were obtained from mice treated with pregnant mare serum and human chorionic gonadotropin, and isolated from cumulus cells by brief exposure to hyaluronidase in PBS. Following isolation, oocytes and eggs were carefully washed in the complete bath medium of Okamoto *et al.*[3] and immediately processed for electrophysiology. Experiments were routinely carried out at room temperature (23–25 °C) in a 1 ml perfusion bath. Similar results were obtained from control experiments carried out at 37 °C. Glass microelectrodes filled with 1.5 M K acetate (resistance 15–30 MΩ) were used in all experiments. Determinations of membrane resistance were made with the aid of a modified bridge circuit[4,5] by a series of brief hyperpolarising pulses of a few mV amplitude.

To investigate whether ACh sensitivity changes throughout oocyte growth, we have examined pre-antral oocytes at an intermediate stage of cell growth. A mean resting potential of -39.3 ± 4.3 mV ($n = 12$) was found in pre-antral oocytes. These cells responded to both high and low ACh doses mainly by membrane hyperpolarisation accompanied by an increase in input membrane resistance. Few cells underwent ACh depolarisation up to -25 and -30 mV (10^{-4}–10^{-2} M ACh), and here a decrease in membrane resistance was observed. Taken together, these findings indicate that ACh sensitivity is a constant feature of dictyate mouse oocytes, at least from intermediate to terminal stages of cell growth. The depolarising ACh response apparently reached a maximum at the end of the cell growth period, that is, with the appearance of the antrum inside the follicle.

In metaphase II oocytes, a mean resting potential of -24.3 ± 1.4 mV ($n = 12$) was observed, in agreement with previous observations[3]. These oocytes responded to ACh with a pattern of membrane potential depolarisation and/or hyperpolarisation similar to that observed in the antral cells.

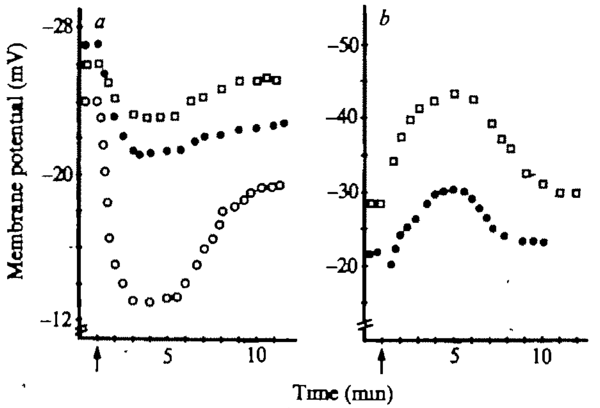

Fig. 2 ACh-elicited depolarisation (*a*) and hyperpolarisation (*b*) in five antral mouse oocytes treated with different doses of ACh. Following ACh application (arrow), a depolarising or hyperpolarising response gradually developed, and the maximum level of response was attained after 2–4 min. Note desensitisation after prolonged exposure of the cells to the drug. *a*: □, 0.5 mM; ●, 2 mM; ○, 7 mM. *b*: □, 10^{-7} M; ●, 2 mM.

Fertilised eggs had a membrane potential (-41.8 ± 3.1, $n = 34$) higher than that of unfertilised oocytes, in agreement with previous observations[6]. Unlike primary and secondary oocytes, fertilised mouse eggs displayed no ACh-elicited responses. ACh doses ranging from 10^{-4} M up to 1.5×10^{-2} M did not elicit any change in membrane potential and input membrane resistance in eggs collected at various times after fertilisation (5–20 h). We have also tested two-cell embryos (membrane resting potential -48.4 ± 2.2 mV, $n = 6$), and here again we did not observe any ACh response. Therefore, the disappearance of cholinergic response is an early event following fertilisation, lasting at least until the two-cell stage of embryonic development. It would be interesting to know whether this disappearance of oocyte ACh sensitivity following fertilisation is directly related to sperm penetration. We favour this hypothesis, as preliminary experiments have shown that responses to ACh are still present in both spontaneously activating and Sr^{2+}-activated mouse eggs (see ref. 7 for parthenogenetic activation of mouse eggs).

We conclude that ACh sensitivity is probably a general feature of vertebrate oocytes, even if in the mouse it is lower than in the amphibians[1]. Mouse oocyte sensitivity to ACh undergoes developmental changes throughout oocyte growth, and it is not grossly affected by the maturation process at ovulation. Finally, ACh sensitivity is a property peculiar to primary and secondary oocytes, as it is lost following fertilisation. This suggests that ACh receptors in vertebrate oocytes may have some specific role in the maturation and/or fertilisation processes.

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Hypothalamic enkephalin neurones may regulate the neurohypophysis

WE have previously reported that significant amounts of immunoreactive (ir)-Leu^5-enkephalin were present in extracts of the neurointermediate lobe of the rat pituitary[1]. Negligible amounts of the pentapeptide were detected in the anterior lobe. In these assays, the concentration of Leu^5-enkephalin in the neurointermediate lobe was higher than in the globus pallidus, the brain region reported to contain the densest enkephalinergic innervation[2]. The high content of (ir)-Leu-enkephalin in the neurointermediate lobe of the pituitary led us to further investigation of its distribution and possible function. We report here that (ir)-enkephalins in the pituitary are concentrated in nerve fibres projecting from the hypothalamus to the pars nervosa and that this pathway may be involved in the regulation of neurohypophysial neurosecretion.

Under a dissecting microscope the rat pituitary could be easily separated into the anterior lobe (pars distalis) and the neurointermediate lobe, and the latter dissected into pars nervosa and the lobules of the pars intermedia. The isolated elements of single pituitaries were each then homogenised and assayed by radioimmunoassay[1,3] for β-endorphin, and Leu^5-enkephalin (Fig. 1). The major portion of (ir)-Leu^5-enkephalin (76%) was found in the pars nervosa. In using a radioimmunoassay for Met^5-enkephalin described elsewhere[4], we found that the pars nervosa contained 1,920 ± 240 pg ($n = 5$; mean ± s.e.m.) of (ir)-Met^5-enkephalin. The ratio of (ir)-Met^5-enkephalin to (ir)-Leu^5-enkephalin was ~3, a similar value to that found in other rat brain areas rich in enkephalins[4]. (ir)-β-endorphin was almost absent from the pars nervosa, but was present in both pars distalis and pars intermedia, as indicated in previous studies[1,5].

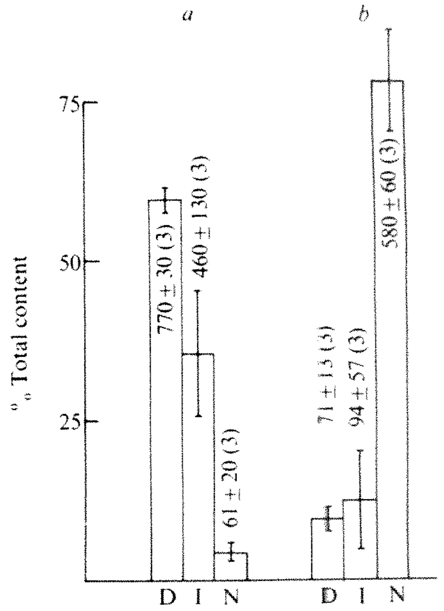

Fig. 1 Pituitaries of Sprague-Dawley male rats (200 g) were dissected under the microscope and pars distalis (D), pars intermedia (I) and pars nervosa (N) were extracted in hot 1 M acetic acid as described in ref. 3, which also contains the complete procedure for the radioimmunoassays of β-endorphin and Leu^5-enkephalin. Dose–response curves of the pituitary extract shows parallelism with synthetic Leu^5-enkephalin. Gel filtration of the extracts on a Bio-Gel P-60 column (50 × 0.7 cm) equilibrated in 50% acetic acid showed that all the (ir)-Leu^5-enkephalin was eluted in a symmetrical peak indistinguishable from that obtained with synthetic Leu^5-enkephalin. Neither vasopressin (AVP or LVP) nor oxytocin crossreact in our Leu^5-enkephalin radioimmunoassay at doses up to 100 g per assay tube. *a*, β-endorphin (ng per pars); *b*, Leu^5-enkephalin (pg per pars).

Table 1 (ir)-Leu^5-enkephalin in neurointermediate lobe after hypothalamic lesions

	pg per lobe (mean ± s.e.m.)
Controls (6)	503 ± 31
Paraventricular nucleus lesions (8)	289 ± 21*
Paraventricular and supraoptic lesions (2)	55

Discrete anodal lesions were made with stainless steel wires, insulated to the tips and stereotaxically positioned in the paraventricular nuclei or in both paraventricular and supraoptic nuclei of male, Sprague-Dawley rats (250 g). Control lesions were placed in the cortex. Seven days after lesioning, radioimmunoassay for Leu^5-enkephalin was carried out on neurointermediate lobes extracted in 1 M acetic acid as described in Fig. 1. All lesion sites were histologically verified. Numbers in parentheses refer to the number of animals tested.

*$P < 0.001$, *t* test.

Using a peroxidase-coupled goat anti-rabbit IgG for indirect immunocytochemistry[6], fine varicose nerve fibres showing Leu^5-enkephalin immunoreactivity were localised in the pars nervosa. These fibres were concentrated on the perimeter of the neural lobe and more or less absent from the centre of the tissue (Fig. 2*b*). Fibres with similar immunoreactivity could be traced in favourable sections through the pars tuberalis (pituitary stalk), and as reported by others, were also present in supraoptic and paraventricular nuclei (ref. 7 and Fig. 2*a*).

The antiserum also stained corticotroph cells of the anterior lobe and all cells of the pars intermedia. As these intermediate lobe cells contain high amounts of β-endorphin, postmortem degradation by peptidases could have given rise to Met^5-enkephalin in sufficient amounts to be detected by our enkephalin antiserum.

After pretreatment with colchicine, (ir)-Leu^5-enkephalin cell bodies were visualised in the paraventricular (Fig. 2*a*) and supraoptic nuclei of the hypothalamus. Varicose fibres from these neurones could be traced through the lateral hypothalamic area, along the course followed by magnocellular neurosecretory axons. Lesioning experiments further suggest that the enkephalinergic cell bodies in the magnocellular nuclei have projections in the pars nervosa. Six days after sectioning the pituitary stalk or lesioning the medial basal hypothalamus, (ir)-enkephalinergic fibres were absent from the pars nervosa.

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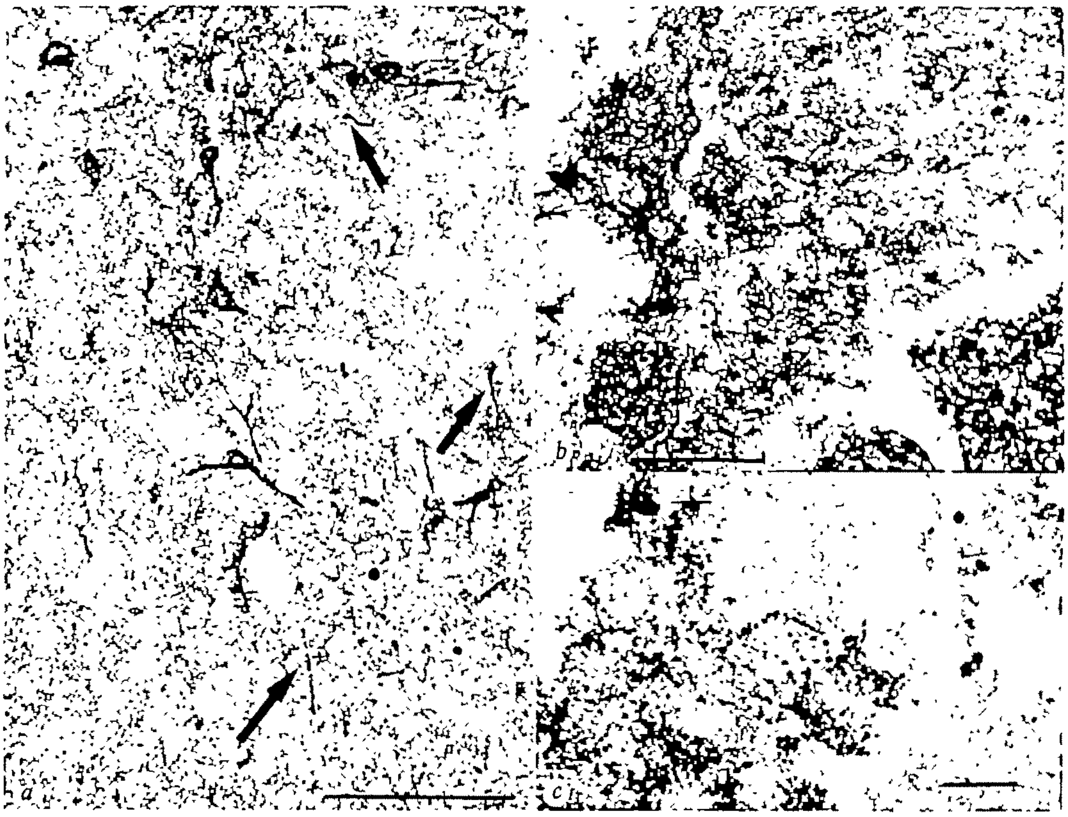

Fig. 2 Photomicrographs of rat hypothalamus (*a*), and neurohypophysis (*b*, *c*) reacted with rabbit anti-Leu^5-enkephalin and a horseradish peroxidase-tagged goat anti-rabbit IgG, as described elsewhere[9]. *a*, After intracerebroventricular colchicine (25 µg, 24 h) neurones in the lateral pole of the paraventricular nucleus exhibit positive immunoreactivity in their dendrites, perikarya and in some finer varicose processes (arrow) presumed to be axons. *b*, Neurohypophysis and intermediate lobe (lower right) of normal rat. Fine varicose immunoreactive fibres can be seen only at the perimeter of the neurohypophysis. Cells of the intermediate lobe show general cytoplasmic immunoreactivity. *c*, Higher magnification view of immunoreactive fibres at the perimeter of the neurohypophysis. Fibres identical to these were seen in occasional patches in Brattleboro rats pretreated with colchicine (24 h, 25 µg), but in untreated Brattleboro rats, no neurohypophysial immunoreactivity was observed. Note that at this higher magnification, the immunoreactive fibres seem to be enveloping groups of non-reactive axons at the perimeter of the neurohypophysis. Calibration bars: *a*, 50 µm; *b*, 25 µm; *c*, 10 µm.

As shown in Table 1, radioimmunoassay measurements confirmed that discrete damage to the paraventricular nucleus resulted in a 40% decrease in the levels of (ir)-Leu^5-enkephalin in neurointermediate lobe extracts. Further damage to the supraoptic nucleus reduced levels to 10% of controls.

The detection of a neuronal pathway between enkephalin-immunoreactive neurones in the magnocellular hypothalamic nuclei and enkephalin-(ir) terminals in the pars nervosa suggests at least two possible functional roles. As with other neurohypophysial hormones, enkephalin could be released as a neurohormone into the bloodstream with vasopressin or oxytocin. Alternatively, enkephalinergic fibres could somehow interact with the secretion of neurohypophysial hormones. Indeed, we find that salt loading decreases (ir)-Leu^5-enkephalin in the neurointermediate lobe (Table 2). Five days after substitution of 2% NaCl for drinking fluid, the vasopressin stores in the neurohypophysis decrease to ~20% of control[8]. In these conditions, we find that (ir)-Leu^5-enkephalin content drops to 37% of control. However, this degree of dehydration did not affect β-endorphin levels in the anterior and intermediate lobes of the pituitary (Table 2). (ir)-Leu^5-enkephalin levels were also not changed in the hypothalamus during this treatment.

Levels of thyrotropin-releasing hormone (TRH) and somatostatin in these extracts were also determined, using radioimmunoassays described elsewhere[9,10]. These two peptides are highly concentrated in the hypothalamus and also in the neurointermediate lobe of the pituitary (ref. 19 and W. Vale, personal communication). In the neurointermediate lobe we found 120±16 pg of (ir)-TRH per lobe ($n = 6$) and 810±220 pg of (ir)-somatostatin per lobe ($n = 6$). Neither pituitary nor hypothalamic levels of TRH and somatostatin were affected by dehydration, indicating that the mechanisms producing the (ir)-Leu^5-enkephalin decrease in the neurohypophysis are peptide specific.

The striking effect of dehydration on the (ir)-Leu-enkephalin content on the neurointermediate lobe led us to examine the distribution of enkephalin in the Brattleboro rat. In this strain the homozygous offspring are polyuric and totally unable to synthesise or secrete vasopressin[8]. When compared with unaffected heterozygote littermate controls, the (ir)-Leu^5-enkephalin levels (Table 2) in the neurointermediate lobe of Brattleboro rats are very low (33% of the heterozygous controls). No differences between homozygous and heterozygous Brattleboros were observed in the (ir)-Leu^5-enkephalin

Table 2 Opioid peptides in pituitary after dehydration

Rat strain	(ir)-β-endorphin ng per lobe (mean ± s.e.m.)		(ir)-Leu^5-enkephalin pg per lobe (mean ± s.e.m.)
	Anterior	Neuro-intermediate	Neuro-intermediate
Sprague-Dawley			
Controls (6)	375±46	661±66	880±60
5 d with 2% NaCl drinking fluid (6)	475±79	647±117	320±80†
Brattleboro			
Controls (*Di*/?) (4)	850±103	705±80	470±19
Polydypsic (*Di*/*Di*) (4)	1,205±98*	1,140±140*	156±26†

In two separate experiments, Sprague-Dawley male (200 g) rats which had received 2% NaCl as drinking fluid for 5 d were compared with their controls which had received plain tap water, and male (120 g) homozygous (*Di*/*Di*) Brattleboro rats were compared with their control littermates (*Di*/?). Radioimmunoassay for β-endorphin and Leu^5-enkephalin was carried out on 1 M acetic acid extracts as described in Fig. 1. Weights of the control Sprague-Dawley anterior and neurointermediate lobe were, respectively, 6.2±0.3 and 0.95±0.07 mg. The 2% NaCl animals had a statistically significantly ($P < 0.01$) decreased weight of their anterior lobe (4.2±0.4) and no statistically significant change in their neurointermediate lobe weight (0.80±0.04).

*$P < 0.05$; †$P < 0.01$, *t* test.

levels of hypothalamus or striatum. (ir)-β-endorphin was slightly elevated in the homozygous Brattleboro rats, both in the anterior and posterior lobes of the pituitary, but was unchanged in the hypothalamus. Immunocytochemical studies of the enkephalin fibres in the pars nervosa of the Brattleboro indicated that the number of enkephalin fibres here are decreased and their distribution and morphology somewhat dystrophic (Fig. 2*c*).

Morphine injections are known to release vasopressin into the bloodstream[11], and opiate receptors are also concentrated in the pars nervosa[12]. Therefore, we propose that these receptors are related to the enkephalinergic innervation of the pars nervosa. Furthermore, because dehydration decreased both the enkephalin and the vasopressin content of the pars nervosa, we propose that pituitary enkephalin fibres may have a role in the regulation of vasopressin or other magnocellular hormonal secretion.

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The granulated peripolar epithelial cell: a potential secretory component of the renal juxtaglomerular complex

THE renal juxtaglomerular complex is conventionally considered to consist of the afferent and efferent glomerular arterioles, the macula densa of the distal tubule, and the interposed polar cushion region[1]. The afferent arteriolar wall contains myoepithelial cells characterised by the presence of multiple secretory-type cytoplasmic granules. It is widely accepted that such granules contain renin[2], the hormone responsible for the production of angiotensin I from plasma renin substrate. We now report the finding of another potential secretory component of the juxtaglomerular complex, raising the possibility of unsuspected functional interactions in this region.

During a study of the influence of salt-balance status on juxtaglomerular morphology in sheep, considerable difficulty was experienced in detecting arteriolar myoepithelial cell granules using Bowie's stain[3] or electron microscopy. However, sections through the vascular pole of many glomeruli revealed the presence of a new, distinctive type of cell which contains multiple cytoplasmic granules closely resembling those of the arteriolar myoepithelial cells. These new cells are in an epithelial position, encircling the origin or polar region of the glomerular tuft (Fig. 1). We have therefore called them 'peripolar cells'. They form junctional complexes with the parietal epithelium of Bowman's capsule on one side and visceral podocytic epithelium on the other; one surface is attached to the basement membrane of Bowman's capsule and the other is directly exposed to the urinary space. Up to four such peripolar cells have been observed surrounding the origin of any one glomerular tuft. The chief morphological characteristic of such cells is the presence of large numbers of closely packed cytoplasmic granules which react positively with methylene blue, the periodic acid-Schiff technique, Brilliant Crystal Scarlet (using Lendrum's Martius-Scarlet Blue stain[4] following fixation in Helly's fluid) and Bowie's stain. Electron microscopically, the granules are membrane-bound, mostly round in shape and contain homogeneous, finely fibrillo-granular electron-dense material (Fig. 2). Typically, most granules range from 100 to 500 nm in diameter. Although most granules in any one cell are of fairly uniform electron density, a few granules display a paler matrix. Granules at the cell periphery have occasionally been observed in the process of discharging their contents into the urinary space. The cytoplasm also contains mitochondria, prominent Golgi membranes, segments of rough endoplasmic reticulum, free ribosomes, and scattered microfilaments and microtubules.

Granulated peripolar cells are usually large in sheep, are sometimes prominent in man, but are less readily found and much smaller in rats and mice. It is most interesting that, thus far, prominent peripolar cells have been found in species with poorly developed afferent arteriolar granulation; in contrast, peripolar cells seem to be relatively smaller in species with abundant arteriolar granulation. The composition of the granules and their function await elucidation. In view of their topographical location, it is almost certain that they participate in juxtaglomerular complex activity. They are ideally situated to release factors directly into Bowman's capsular space, perhaps triggered by electrolyte changes in the glomerular ultrafiltrate, or by variations in afferent or efferent arteriolar calibre (leading to alterations in the diameter of the polar region of the glomerular tuft), or in response to diffusible mediators released from other components of the complex. Factors released from

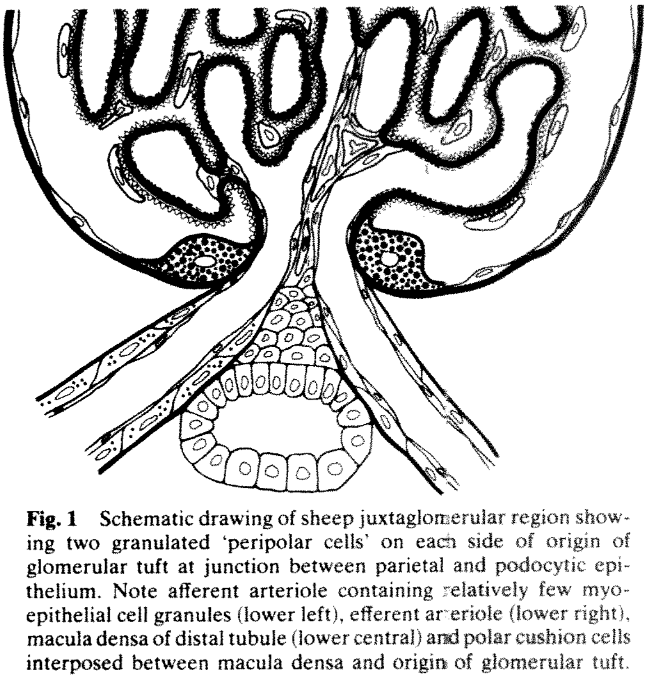

Fig. 1 Schematic drawing of sheep juxtaglomerular region showing two granulated 'peripolar cells' on each side of origin of glomerular tuft at junction between parietal and podocytic epithelium. Note afferent arteriole containing relatively few myoepithelial cell granules (lower left), efferent arteriole (lower right), macula densa of distal tubule (lower central) and polar cushion cells interposed between macula densa and origin of glomerular tuft.

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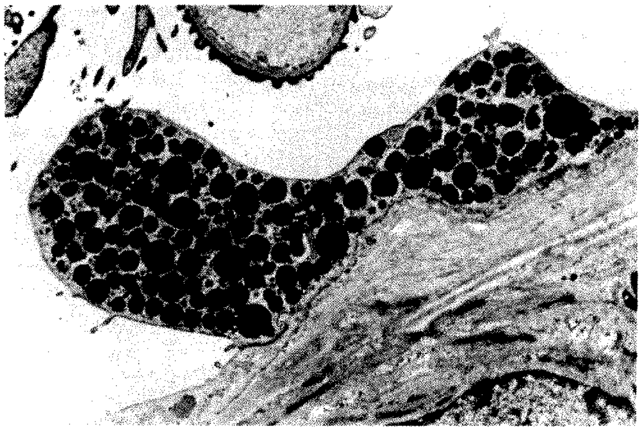

Fig. 2 Electron micrograph of sheep 'peripolar cell' containing multiple electron-dense cytoplasmic granules; its free surface is directly exposed to Bowman's capsular space. Note portion of juxtaglomerular arteriole (lower right) and glomerular tuft capillary (upper central). ×5,000.

peripolar cells could pass into the tubular fluid and be involved in the modulation of secretory or resorptive functions of tubular cells. There has been much study directed towards determining the factors controlling proximal tubular sodium reabsorption and glomerulotubular balance[5-7]. One school of thought holds that a previously unrecognised hormone is involved in these processes. It has recently been reported that proximal tubular fluid contains a factor which mediates increased volume reabsorption in the proximal tubule in response to increased glomerular filtration rate[8]; it was suggested that such increased volume reabsorption results from increased proximal tubular sodium reabsorption[8]. The proximal tubular fluid factor responsible for these effects was not present in an artificial ultrafiltrate of plasma[8], indicating that it is added to the tubular fluid after filtration of plasma across the glomerular capillary wall. It is possible that the peripolar cell is the source of such a factor, thereby having a key role in glomerulotubular balance and electrolyte homeostasis.

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T-cell hybrids bear Fcγ receptors and secrete suppressor immunoglobulin binding factor

PROGRESS in the study of antibody structure and function came from the establishment of plasmocytomas and B-cell hybrids[1] which produce large amounts of monoclonal antibodies. Another set of immune molecules, the T-cell factors regulating antibody production[2], have not previously been extensively characterised, mainly because of the lack of homogenous and reproducible sources. The use of T-cell hybrids[3-5] which would produce large amounts of a given factor would therefore be very useful for the characterisation of these molecules. Among the T-cell factors regulating antibody production, Gisler and W.H.F.[6] have described an immunoglobulin binding factor (IBF) which binds to IgG, not to IgM and suppresses antibody production to T-dependent and T-independent antigens nonspecifically *in vitro*. IBF is the soluble form of the T-cell Fcγ receptor[7] which is expressed on non antigen-specific suppressor T cells[8,9]. As the Fcγ receptor is a marker of a set of suppressor T cells, one would expect that selection of T-cell hybrids for the expression of this receptor may lead to selection of T-cell lines producing permanently non antigen-specific suppressor factor. We report here the establishment of T-cell hybrids which express Fcγ receptors and produce suppressor IBF.

T1 hybrid cell line and clones were obtained by fusion of BW 5147 T lymphoma cells (hypoxanthine, aminopterin and thymidine (HAT) sensitive, $H\text{-}2^k$ Thy-1.1 positive, Fcγ receptor negative) with spleen cells from C57BL/6 ($H\text{-}2^b$) mice primed with sheep red blood cells (SRBC). The T2C2 and T2D4 cell lines were obtained by fusion of the BW 5147 T lymphoma cells with B10.BR alloantigen-activated T cells ($H\text{-}2^k$, Thy-1.2 positive and 40% Fcγ receptor positive). Cells were fused using polyethylene glycol (PEG) as a fusion agent (Table 1). After distribution in microplates and two weeks of selection in HAT medium, hybrid populations were grown in RPMI 1640 medium (Eurobio) containing 10% fetal calf serum (FCS) (Eurobio) (RPMI FCS). The hybrids were selected for the presence of membrane Fcγ receptors by a rosette technique and tested for the expression of H-2, Thy-1 and Lyt alloantigens by microcytotoxicity assays. Rosette determinations were carried out at 4 °C using sheep erythrocytes (E) sensitised with IgG (EAIgG) or IgM (EAIgM) rabbit anti-Forssman antibodies[7]. The clones

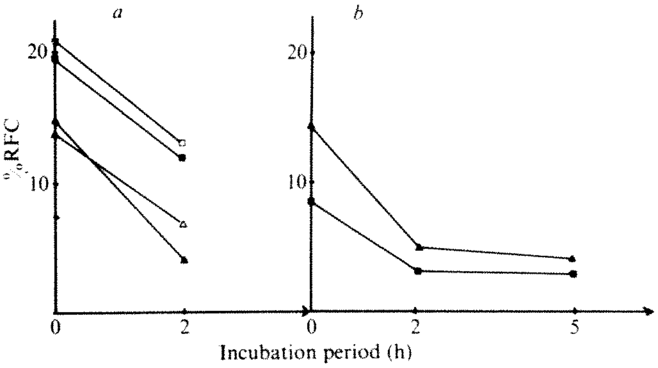

Fig. 1 Release of Fc receptors from T-cell hybrids. Hybrid cells were incubated from 2–5 h at 37 °C at a concentration of 2×10^6 cells per ml and tested for their capacity to form rosettes with EAIgG. Rosette determinations were performed at 4°C by mixing 5×10^5 hybrid cells with 10^7 IgG-sensitised erythrocytes in 0.5 ml BSS. After centrifugation at 1,000 r.p.m. for 10 min at 4°C, and gentle resuspension, rosettes were counted. *a*, □, T_1C1E_9; ■, T1; ▲ T1ClE5; △ T1ClE4; *b*, ▲, T2C2; ■ T2D4. RFC, Rosette-forming capacity.

Table 1 Karyotic analysis and cell surface markers of T-cell hybrid lines

Hybrid cell lines*	Parental populations†		Average no. of chromosomes‡	Cell surface markers§
T1	AKR T lymphoma BW5147 ($H-2^k$, Thy-1,1)	× Spleen cells from C57BL/6 mice immunised with SRBC ($H-2^b$, Thy-1.2, Lyt-1.2, Lyt-3.2)	53	$H-2^b$, $H-2^k$, Thy-1,2, Thy-1,1, Lyt-3,2
T2C2 and T2D4	AKR T lymphoma BW5147	× B10.BR *in vivo* alloantigen-activated T cells ($H-2^k$, Thy-1.2)	51	$H-2^k$, Thy-1.1, Thy-1.2

* Fusion was performed by adding 1 ml of 50% PEG (w/v) 1500 in serum-free RPMI to a pellet of a mixture of 10^8 spleen cells and 10^7 BW5147 cells[3]. After 1 min at 37°C, the suspension was gradually diluted to 10 ml (in 5 min) with serum-free RPMI 1640. After centrifugation RPMI FCS was added and cell suspension was distributed in 0.2 ml microplates (Falcon no. 3040) at a concentration of 2×10^6 cells per well and grown in selective HAT medium in a CO_2 incubator (5% CO_2) in air). Two weeks later, selective medium was removed and replaced by RPMI FCS.

† Spleen cells of C57BL/6 mice were collected 5 days after immunisation by intraperitoneal injection of 10^8 SRBC. Alloantigen-activated B10.BR T cells were taken from spleens of lethally irradiated (850 rad) BALB/c mice reconstituted 5 d before by intraveneous injection of 10^8 B10.BR thymocytes.

‡ The average number of chromosomes of BW5147 was 43. In each experiment 15 to 21 mitoses were observed.

§ The hybrid cell lines were negative for cell surface Ig as revealed by immunofluorescence. The detection of other cell surface markers was performed by a microcytoxicity technique using guinea pig serum as a source of complement. 100% cells of T1 hybridoma were killed by C3H anti-C57BL/6 (anti-$H-2^b$) antiserum up to 1/80 dilution, by C57BL/6 anti-C3H (anti-$H-2^k$) antiserum up to 1/160 dilution, by AKR anti-C3H (anti-Thy-1.2) antiserum up to 1/40 dilution, by C3H anti-AKR (anti-Thy-1.1) antiserum up to 1/20 dilution and by C58 anti-C58.CE Lyt-3.2 (anti-Lyt-3.2) antiserum up to 1/20 dilution. It was resistant to lysis by C3H anti-C3H.CE Lyt-1.2 (anti-Lyt-1.2) antiserum. 100% cells of T2C2 and T2D4 were killed by C57 anti-C3H (anti-$H-3^k$) antiserum up to 1/160 dilution, by C3H anti-AKR (anti-Thy-1.1) antiserum up to 1/20 dilution, and by AKR anti-C3H (anti-Thy-1.2) antiserum up to 1/20 dilution.

T1ClC5, T1ClE4 and T1ClE9 from the Fcγ receptor-positive T1 hybrid were obtained by distribution of individual cells in microplates and were grown in RPMI FCS.

In a first series of experiments we established that T1 and T2 are T-cell hybrids. As shown in Table 1, their number of chromosomes (50–54) is higher than the number of chromosomes of the BW 5147 lymphoma (41–45), and they express H-2 and Thy-1 alloantigens of both parental strains and no cell surface immunoglobulins. In addition, T1 hybrid cells were found to be positive for Lyt-3.2 antigen which is known to be a marker of cytotoxic and suppressor T cells and to be negative for Lyt-1.2 antigen. With regard to the presence of Fcγ receptors, T1 hybrid, its clone derivatives (T1ClC5, T1ClE4, T1ClE9) and the several T2 hybrid populations (T2C2, T2D4 and five others) showed a permanent (over 12 months) capacity to form rosettes with EAIgG, while the parental BW cell line was negative. These rosettes detect specifically the Fcγ receptors as: (1) 100% are inhibited by aggregated rabbit and mouse IgG and not by aggregated $F(ab')_2$ fragments of rabbit IgG or by mouse IgM; and (2) no rosettes were found with E or EAIgM (data not shown).

In previous studies we have shown that, when activated T cells are incubated at 37 °C, the Fcγ receptors disappear from the cell surface and suppressor IBF appears in the supernatant[10]. We therefore tested whether hybridomas would exhibit a similar phenomenon. When taken from the cultures and washed, 6–35% of cells of the different hybrid cell lines could form EAIgG rosettes depending on the phase of the culture. As shown in Fig. 1, after incubation of the hybrid cells for 2–5 h at 37°C at a concentration of 2×10^6 cells per ml in serum-free BSS, the number of EAIgG rosette-forming cells (EARFC) was much decreased. This was not due to death of Fcγ receptor-positive cells as viability remains constant throughout the incubation period, but rather suggests that the hybrid cell lines, like the activated T cells[7], may release Fcγ receptors into the medium. To determine if suppressor IBF is produced by the hybrid cells in these conditions, supernatants of 2×10^6 cells incubated for 2 h at 37 °C in serum-free BSS were tested for the presence of a suppressor factor which could be retained on IgG immunoadsorbents. Table 2 shows representative experiments. In most cases, crude supernatants of the hybrid cell lines (T1ClC5, T1ClE4, T1ClE9, T2C2 and T2D4) suppressed *in vitro* antibody production to SRBC and this suppressive activity was recovered in the acid eluates of IgG immunoadsorbents but not in the effluents. In one case (T1), even if no suppressive activity could be found in the crude supernatant, the acid eluate and not the effluent strongly inhibited the PFC response. As a control, neither the crude supernatants nor their acid eluates of BW 5147 parental cell line exerted suppressive activity. As the acid eluates contain only molecules which bind to IgG—IBF by definition—and the effluents are specifically devoid of these molecules, it is possible to state that the Fcγ receptor-positive T-cell hybrids produce suppressor IBF.

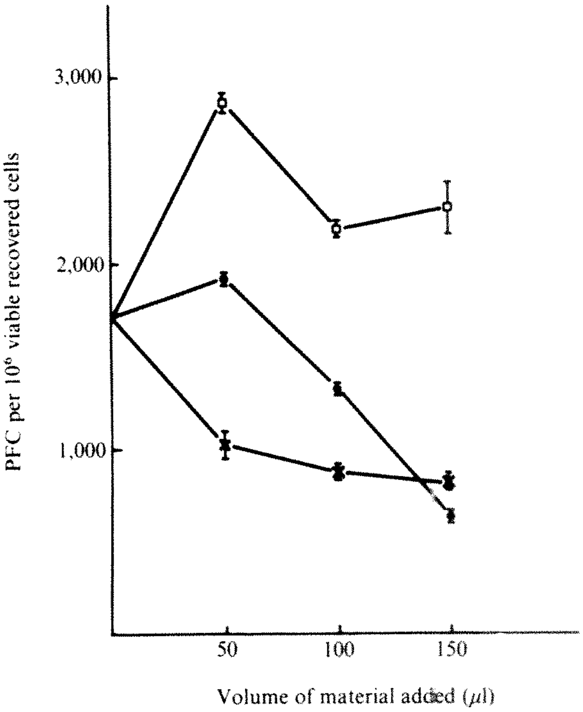

Fig. 2 Titration of suppressive IBF produced by T1 hybrid cells. Graded doses of crude supernatants (●), effluents (□) and acid eluates (×) from IgG immunoadsorbents were added 48 h after antigen to spleen cell cultures of C57BL/6 mice stimulated by SRBC, using a technique in tubes[6]. Direct PFC were measured at day 5 by the local haemolysis technique in liquid medium[11]. Each culture was made in duplicate and results are expressed as PFC per 10^6 viable recovered cells.

The amount of suppressor factor produced by the hybrids is similar to the amount of suppressive IBF produced by activated T cells, as when supernatants and acid eluates from activated T cells or hybrid cells were prepared in the same way and tested at the same dose, no significant differences could be detected (Table 2). To titrate the suppressor factor produced by hybrid cells more precisely, graded doses of supernatants, effluents and acid eluates from IgG immunoadsorbents were added to spleen cells stimulated with SRBC. Figure 2 shows a representative experiment using T1 hybrid. A dose-dependent inhibition of antibody production was obtained with supernatant and acid eluate, while no suppression was observed with the effluents. Similarly to T-cell-produced IBF[6], 100% inhibition was never found, but 60–70% were obtained with small doses of acid eluate and high doses of crude supernatant, indicating enrichment in suppressive activity. The binding of suppressor factor

Table 2 Production of suppressive IBF by T1, its clones derivatives and T2 hybrid cell lines

Material added to cultures of spleen cells stimulated by SRBC*	T1 PFC/10^6 cells	T1 % In	T1ClC5 PFC/10^6 cells	T1ClC5 % In	T1ClE4 PFC/10^6 cells	T1ClE4 % In	T1ClE9 PFC/10^6 cells	T1ClE9 % In
None	18,975±1380	—	23,064±1726	—	23,064±1726	—	23,064±1726	—
Supernatant†	13,129±96	31	14,950±1150	35	9,200±767	56	9,009±384	61
Effluent from IgG-coated Sepharose beads‡	21,850±383	0	ND	—	ND	—	ND	—
Acid eluate from IgG-coated Sepharose beads‡	8,740±575	54	10,022±1315	57	7,962±885	67	8,913±239	61

Material added to cultures of spleen cells stimulated by SRBC*	T2C2 PFC/10^6 cells	T2C2 % In	T2D4 PFC/10^6 cells	T2D4 % In	BW PFC/10^6 cells	BW % In	Activated cells PFC/10^6 cells	Activated cells % In
None	2,597±245	—	2,597±245	—	6,210±190	—	26,105±6731	—
Supernatant†	623±144	76	1,186±36	54	8,881±64	0	11,131±945	57
Effluent from IgG-coated Sepharose beads‡	2,714±322	0	2,185±460	16	ND	—	ND	—
Acid eluate from IgG-coated Sepharose beads‡	1,093±173	58	1,416±266	45	5,842±129	6	1,0570±398	60

* 8×10^6 spleen cells from B6D2F1 mice were cultured in RPMI 1640 supplemented with 10% FCS (Rehatuin–Rehcis), 1% horse serum and antibiotics (penicillin, streptomycin) in Falcon plastic tubes for 5 d in a CO_2 incubator (5% CO_2 in air)[6]. SRBC (3×10^6 cells) were added at day 0, 100 μl of materials were added 48 h later and direct PFC were enumerated at day 5 by the liquid local haemolysis technique[11]. PFC are expressed per 10^6 viable recovered cells.

† Hybrid cells or activated T cells were washed at 4 °C in BSS and incubated at a concentration of 2×10^6 cells ml^{-1} for 2 h at 37 °C in serum-free BSS. Cell-free supernatants were taken after centrifugation at 200*g* for 10 min.

‡ Cyanogen bromide-activated Sepharose beads (Pharmacia) were coupled with 5 mg ml^{-1} of rabbit IgG (Miles, Eurobio) in sodium bicarbonate buffer, 0.1 M *p*H 8 (ref. 12). For immunoadsorption, a batch technique was used: 2 volumes of supernatants were mixed with 1 volume of Sepharose beads for 2 h at 22 °C, and the effluents were recovered after centrifugation. After washings in phosphate buffer, 0.02 M *p*H 7, the beads were incubated in 0.2 M glycine HCl *p*H 2.8 buffer and the eluates were recovered by centrifugation and neutralised to *p*H 7. Effluents and eluates were concentrated to half starting volume, dialysed against BSS, and sterilised on 0.22 μm Millipore filter.

‖ Alloantigen-activated T cells were prepared as described in legend to Table 1.

ND, Not determined. % In, % inhibition. Thirty per cent inhibition was considered significant.

specifically to IgG and its non-antigen specificity is shown in Table 3. First, in experiment 1, crude supernatant of the T1 hybrid cell line inhibits 21% of the plaque-forming cell (PFC) response, and all the suppressive activity is present in the acid eluate of an IgG immunoadsorbent and not in the effluent. By contrast, when the same supernatant is applied to an IgM immunoadsorbent, suppressive activity is recovered in the effluent and not in the eluate, indicating that the suppressor factor binds specifically to IgG. As T1 hybrid was prepared with spleen cells sensitised with SRBC, it was necessary to rule out the presence of an antigen-specific suppressor factor in the eluates. Experiment 2 in Table 3 shows that the suppressive activity found in acid eluates from IgG immunoadsorbents could not be adsorbed on SRBC, demonstrating that IBF produced by T-cell hybrids as well as IBF produced by activated T cells behave as a non-antigen-specific suppressor factor.

T-cell hybrids have already been obtained by several laboratories[1–5] but in most cases, except for one hybrid producing an antigen-specific suppressor factor[13], immunological functions of parental spleen cells were lost after fusion. A recent report by Taniguchi and Miller[14] describes several T-cell hybridomas obtained by using parental spleen cells enriched for I-J subregion markers and containing specific and nonspecific suppressor factors. However, suppressive activity was lost after 3 months of culture. The present work shows that selection of T-cell hybrids for the presence of Fcγ receptors leads to the establishment of lines which produce permanently (over 12 months) non-antigen-specific suppressor factor. Also, some T lymphomas have been shown to permanently produce immunoregulatory molecules[15] and in particular, Fc receptors[16,17]. However, first, these molecules are of tumour and not T-cell origin and may not be identical to physiological products, and second, hybridisation of a given lymphoma with selected T-cell subpopulations may lead to T-cell lines producing various factors which could be compared on a biochemical basis and whose eventual differences could not be due to different origin of the tumour cells. These T-cell lines may be very useful for the characterisation of factors and for *in vivo* studies.

Table 3 Production of an IgG binding, non-antigen-specific, suppressor IBF by T1 hybrid cell line

Material added to cultures of spleen cells stimulated by SRBC*	PFC per 10 cells	% Inhibition
Expt 1		
None	6,210±190	—
Supernatant	4,910±152	21
Effluent from IgC-coated Sepharose beads	5,485±531	12
Acid eluate from IgG-coated Sepharose beads	2,492±192	60
Effluent from IgM-coated Sepharose beads*	3,368±411	46
Acid eluate from IgM-coated Sepharose beads*	5,134±1,355	17
Expt 2		
None	3,283±293	—
Acid eluate from IgG-coated Sepharose beads	2,058±80	37
Acid eluate from IgG-coated Sepharose beads adsorbed with SRBC‡	2,052±166	37

* See Table 2 legend for details.

‡ One volume of packed SRBC were incubated with 3 volumes of eluate for 1 h at 4°C and centrifuged at 2,000 r.p.m. before use.

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Novel α_2-adrenoreceptors primarily responsible for inducing human platelet aggregation

BLOOD PLATELETS isolated from humans, in contrast to those from other mammalian species, aggregate on exposure to adrenaline or noradrenaline[1]. Studies using α antagonists have shown that this effect results from occupancy of an α adrenoreceptor[2,3]. However, Jakobs has reported that selective α agonists such as clonidine and phenylephrine bind with high affinity to the platelet α adrenoreceptor[4,5], but fail either to induce an aggregation response[6] or to inhibit platelet adenylate cyclase[6], in contrast to the effects of natural agonists such as adrenaline[1,7]. Instead, such α agonists seem to act as antagonists at the platelet adrenoreceptor[6]. α Adrenoreceptors in other tissues have been classified as α_1 (postsynaptic) or α_2 (presynaptic) on the basis of their affinities for various selective α agonists and antagonists[8]. Jakobs[6] has, however, interpreted his data on the human platelet adrenoreceptor as indicating the existence of a third category of α adrenoreceptor at which only the natural catecholamines are agonists. We report here that we have confirmed the observation that clonidine and phenylephrine are ineffective in inducing aggregation of human platelets and act as inhibitors of the response to adrenaline. However, further examination has revealed that the effects of these selective α agonists differ in certain important respects from those of selective α antagonists, thus requiring substantial modification of the thesis advanced by Jakobs[6].

Figure 1 shows the effect of clonidine and of yohimbine, a selective α_2 antagonist[9,10], on the aggregation response of human platelets to ADP, vasopressin and serotonin. Addition of clonidine 15 s before each of these agonists causes marked stimulation of both phases of the response. Stimulation of the response is also observed if clonidine is added before other agonists, such as collagen, thrombin or arachidonate. From the detailed dose–response curve (Fig. 1*a*) the concentration of clonidine which gives half-maximal stimulation of aggregation induced by ADP is 0.6 μM, in agreement with the concentration of this selective α agonist which gives half-maximal inhibition of the primary response to adrenaline (0.6 μM). In contrast, yohimbine, which is also an effective inhibitor of aggregation induced by adrenaline (Fig. 2*a*), has little effect on aggregation induced by most other agonists (compare Fig. 1*b* and *c*) at concentrations which completely inhibit the response to adrenaline, and notably fails to stimulate these responses. The only exception is the response to serotonin where the extent of inhibition by yohimbine is rather more marked (Fig. 1*d*). However, yohimbine is an effective inhibitor of the stimulation by clonidine of the response to other agonists (such as ADP, vasopressin, serotonin)(Fig. 1*b–d*). The concentration of yohimbine required for half-maximal inhibition of stimulation by clonidine of the response to ADP (2.5 μM) is, however, significantly greater than that which causes half-maximal inhibition of the primary phase of the response to adrenaline (0.5 μM).

Similar studies have been carried out using phenylephrine and the selective α_1 antagonist, indoramin[12,13]. Phenylephrine added 15 s before platelet agonists other than adrenaline also

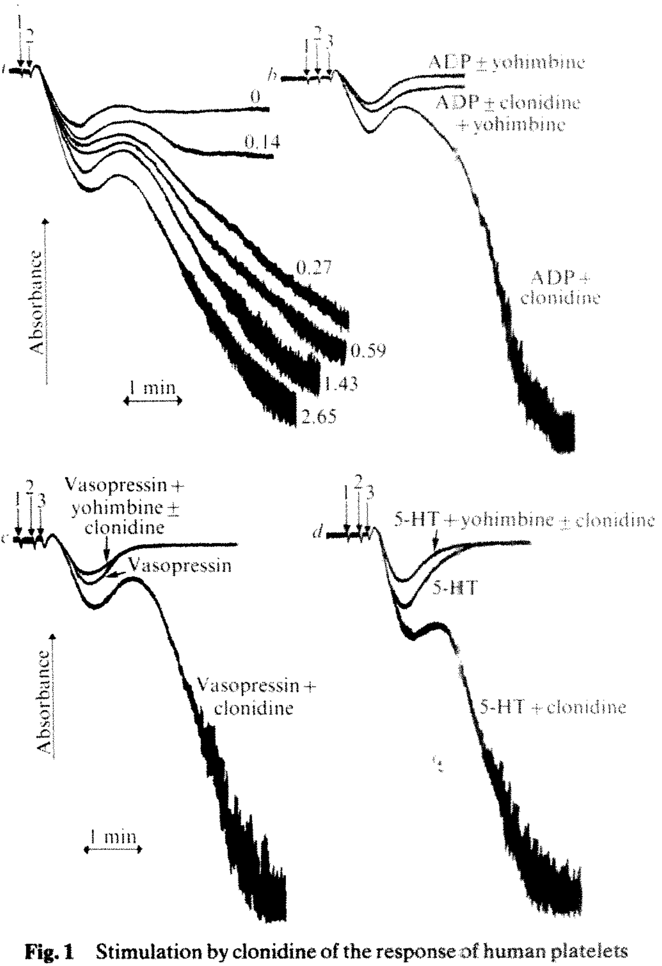

Fig. 1 Stimulation by clonidine of the response of human platelets to ADP, vasopressin and serotonin (5-HT) and the inhibition of this effect by yohimbine. Platelet-rich plasma was obtained from drug-free donors using 0.1 volumes of acid-citrate dextrose (0.1 M citrate) as anticoagulant, and platelet aggregation was monitored using a Payton dual-channel aggregometer as described previously[11]. Additions were as folllows: *a*, at 1, clonidine at the concentrations (μM) indicated by the numbers on the traces; at 2, 1.25 μM ADP; *b*, at 1 when indicated, 5 μM yohimbine, at 2 when indicated, 5.7 μM clonidine, at 3, 0.75 μM ADP; *c*, at 1 when indicated, 10 μM yohimbine, at 2 when indicated, 5.7 μM clonidine, at 3, 0.046 nM vasopressin; *d*, at 1 when indicated 10 μM yohimbine, at 2 when indicated, 5.7 μM clonidine, at 3, 1 μM serotonin. Absorbance is in arbitrary units.

causes a stimulation of the response to these agonists which is similar in magnitude to that observed in the presence of clonidine (Fig. 1). This is shown in Fig. 3 for ADP, thrombin and arachidonate. The concentration of phenylephrine which gives half-maximal stimulation of the response to ADP (30 μM)(Fig. 3*a*) is in reasonable agreement with that which gives half-maximal inhibition of the response to adrenaline (25 μM), although we did not obtain complete inhibition of the adrenaline response by phenylephrine at concentrations where the effect is specific. In contrast, indoramin fails to stimulate the response of human platelets to ADP or thrombin over a range of concentrations which causes inhibition of the response to adrenaline (Fig. 3*b*, *c*). The concentration of indoramin required to cause 50% maximal inhibition of the adrenaline response is 42 μM (Fig. 2*b*). Although 150 μM indoramin causes complete inhibition of the response to adrenaline the effect may represent a nonspecific action, as this concentration also causes complete inhibition of the response to ADP. Specific inhibition of the adrenaline response is only observed at indoramin concentrations <50 μM. Both yohimbine and indoramin selectively inhibit phenylephrine stimulation of the response to ADP (Fig. 3*b*), The concentrations of these antagonists required to give half-maximal inhibition of the stimulated response are 27 and 2 μM, respectively. Indoramin also selectively blocks phenylephrine-induced stimulation of the response to thrombin (Fig. 3*c*).

The stimulation of the response of human platelets to agonists such as ADP, vasopressin, serotonin and thrombin which results from the addition of the specific α agonists (Figs 1, 3) resembles that reported previously for rat platelets in which phenylephrine was found to enhance the response to ADP[14]. The effect in human platelets is quantitatively similar to that observed when a non-aggregating concentration of adrenaline is added before any one of these agonists[2,15]. As selective α antagonists fail to stimulate the response of human platelets to agonists other than adrenaline but act as inhibitors with respect to adrenaline itself, selective α agonists such as clonidine and phenylephrine seem to interact as partial agonists at the platelet adrenoreceptor rather than as antagonists as suggested by Jakobs[6]. This situation, although unusual, is not unique, as other systems have been described in which clonidine at least, seems to act as a partial agonist[16].

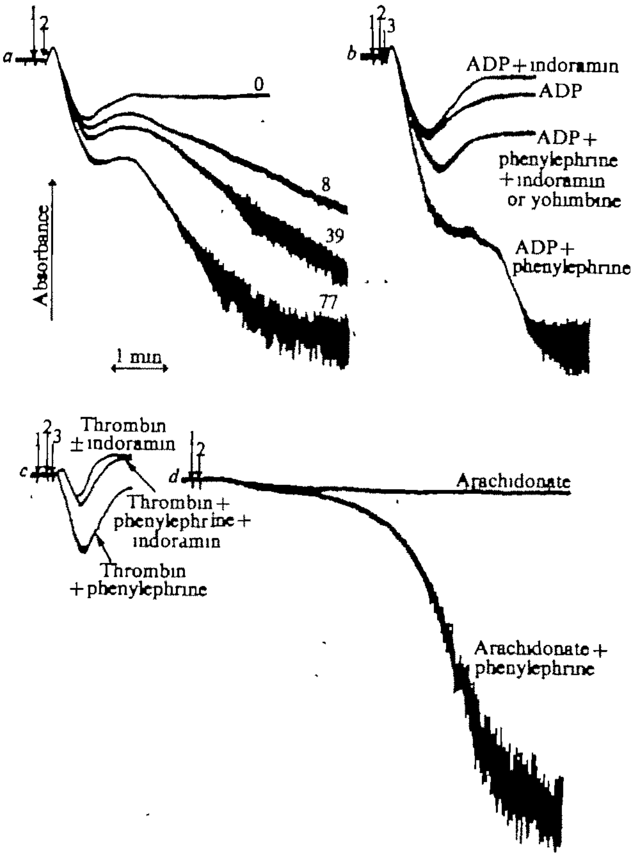

Fig. 3 Stimulation by phenylephrine of the response of human platelets to ADP, thrombin and arachidonate and the inhibition of this effect by yohimbine and indoramin. Platelet aggregation was monitored as described for Fig. 1. Additions were as follows: *a*, at 1, phenylephrine at the concentrations (μM) indicated by the numbers on the traces; at 2, 1.25 μM ADP, *b*, at 1 when indicated, 10 μM indoramin or 40 μM yohimbine, at 2 when indicated, 77 μM phenylephrine and at 3, 1.5 μM ADP; *c*, at 1 when indicated, 5 μM indoramin, at 2 when indicated, 77 μM phenylephrine, at 3, 0.28 units thrombin; *d*, at 1 when indicated, 38.5 μM phenylephrine; at 2, 0 4 μM arachidonate.

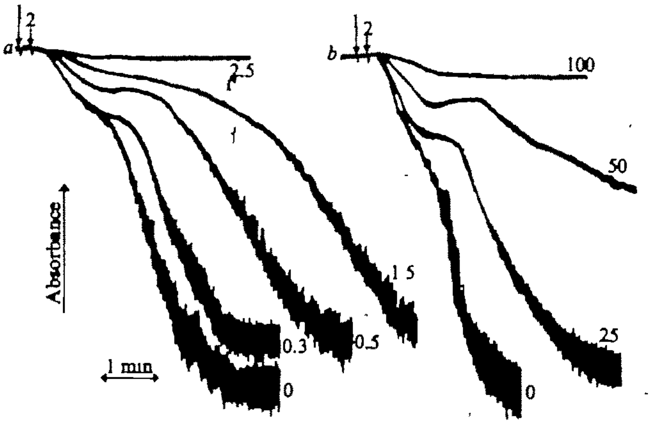

Fig. 2 Inhibition by yohimbine and indoramin of the response of human platelets to adrenaline. Platelet aggregation was monitored as described for Fig. 1. Additions were as follows: *a*, at 1, yohimbine at the concentrations (μM) indicated by the numbers on the traces, at 2, 5 μM adrenaline; *b*, at 1, indoramin at the concentrations (μM) indicated by the numbers on the traces; at 2, 5 μM adrenaline.

Our data also provide insight into the nature of the adrenoreceptors on human platelets that are responsible for initiation of the aggregation response. As yohimbine is two orders of magnitude more potent than indoramin as an antagonist of aggregation induced by adrenaline (Fig. 2), this latter response seems to be mediated by α_2 adrenoceptors. A similar conclusion is applicable to the action of clonidine as a partial agonist in stimulating the response to agonists other than adrenaline, although in this case, blockade by yohimbine occurs over a range of concentration significantly higher than that required for inhibition of the response to adrenaline (Figs 1, 2). In contrast, indoramin at concentrations <50 μM has little effect as an inhibitor of the clonidine-stimulated responses, as would be expected from the relative efficiency of the two antagonists on the response to adrenaline (Fig. 2). However, the pattern of inhibition by indoramin and yohimbine is exactly reversed for phenylephrine stimulation of the response to agonists other than adrenaline: In this case, complete blockade of the stimulated response by indoramin occurs at a concentration (5–10 μM) which has little, if any, effect on aggregation induced by adrenaline (Figs 2*b*, 3*b*, *c*). Blockade by yohimbine of the phenylephrine stimulation is, in contrast, less effective than that caused by indoramin and occurs only at concentrations of yohimbine well in excess of those required for inhibition of the responses to adrenaline or clonidine. This pattern of inhibition suggests that, in contrast to adrenaline or clonidine, phenylephrine is exerting its effects through α_1 adrenoreceptors which are, however, less effective in inducing aggregation and make only a minor contribution to the response to the natural agonist.

Both our data and those of Jakobs[6] seem, therefore, to be satisfied by a postulate which suggests that human platelets carry both α_1 and α_2 adrenoreceptors at which phenylephrine and clonidine, respectively, act as partial agonists. The α_2 adrenoreceptor is identified as being primarily responsible for mediating the response to the natural agonist. This conclusion seems to agree with the nature of the response of human platelets to adrenaline that involves secretion as well as aggregation[2,8]. The proposal by Jakobs[6] that the platelet carries a unique species of adrenoreceptor does not seem warranted by the data now available.

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Amines inhibit the clustering of α_2-macroglobulin and EGF on the fibroblast cell surface

α_2-MACROGLOBULIN (α_2M), insulin and epidermal growth factor (EGF) have a common mechanism for internalisation[1]. We have proposed a model for this mechanism[1-3] in which the ligands bind to diffusely distributed receptors followed by the collection of the mobile ligand–receptor complexes over coated pits. These coated pits pinch off from the plasma membrane to form endocytic vesicles. The diffuse initial distribution of the α_2M receptors has been shown by binding α_2M to fixed cells and observing the location of α_2M by an electron microscopic immunocytochemical method[2]. The mobility of the ligand–receptor complexes has been demonstrated using fluorescence photobleaching recovery[4], and the clustering of such complexes has been demonstrated by fluorescence microscopy[1,3,5]. Electron microscopic immunocytochemical localisation has been used to show that the clustering occurs over coated pits which pinch off to form endocytic vesicles[2]. The mechanism proposed for the endocytosis of α_2M, insulin and EGF is similar to that described for the internalisation of low density lipoprotein (LDL), except that unoccupied LDL receptors may cluster in coated pits before binding LDL[6]. It seems likely that many molecules, including other polypeptide hormones and the lysosomal hydrolases[7] which bind to specific receptors on the cell surface, are internalised by a similar mechanism. To determine the role of clustering and internalisation in hormone action it would be useful to have an agent that inhibits these processes. It has been found that the internalisation of lysosomal enzymes is noncompetitively inhibited by some amines and ammonia (E. Neufeld, personal communication). It has also been reported that ammonia prevents the internalisation of diptheria toxin by HeLa cells[8]. In view of these effects, we examined the possibility that amines and ammonia would block the clustering of α_2M and EGF, and we report here that these agents are effective in blocking clustering and internalisation.

When rhodamine-labelled α_2-macroglobulin (R-α_2M) is added to the culture medium of Swiss 3T3-4 cells, the fluorescent emission from the R-α_2M is observed in a punctate distribution on the cells[5] (Fig. 1*a*) as a result of clustering and endocytosis[1,2]. In contrast to the punctate fluorescence seen in Fig. 1*a*, R-α_2M added after a brief incubation with methylamine forms a diffuse pattern of fluorescence on the cells (Fig. 1*b*). No inhibition of clustering was noted at amine concentrations below 0.1 mM, and the inhibition of clustering is complete at 10–30 mM methylamine hydrochloride.

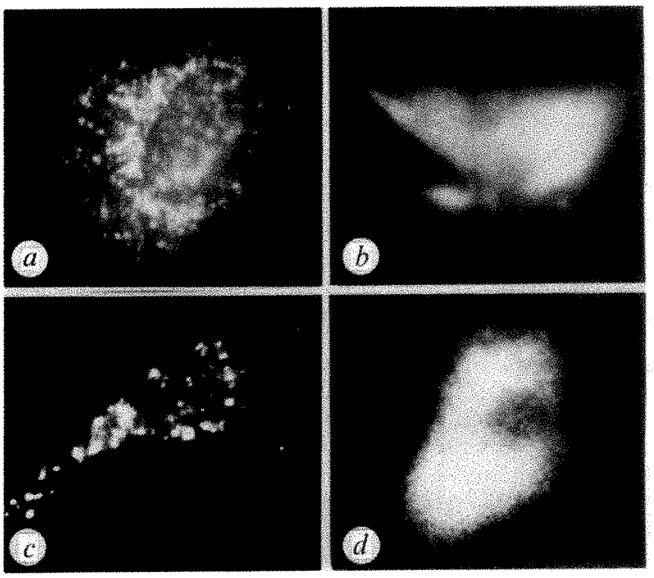

Fig. 1 Inhibition of α_2M clustering. *a*, Swiss 3T3-4 cells were incubated for 20 min at 37 °C in Dulbecco–Vogt's modified Eagle's medium containing 30 μg ml^{-1} R-α_2M. *b*, Cells were incubated with 10 mM methylamine hydrochloride for 20 min at 37 °C and 30 μg ml^{-1} R-α_2M was then added for a further 20 min incubation at 37 °C. In *c*, 30 mM methylamine was present before and during the 20 min incubation with R-α_2M. The cells were then rinsed with fresh medium and incubated for 20 min at 37 °C. This resulted in a punctate fluorescence pattern similar to that in *a*. When methylamine was present for the final incubation (not shown), the fluorescence remained diffuse as in *b*. *d*, Cells were incubated in medium without Ca^{2+}, containing 1 mM Mg^{2+} and 1 mM EGTA, for 5 min at 37 °C. 30 μg ml^{-1} R-α_2M was then added, and the cells were incubated for 30 min at 37 °C. After the indicated incubations, the cells were rinsed and fixed with 2% formaldehyde in Dulbecco's phosphate-buffered saline for 1 min at 23 °C. The fluorescence was recorded on videotape using a silicon intensifier target television camera[5], and photographs were taken from the video monitor. Magnification, ×678.

Ammonium acetate and the hydrochloride salts of methylamine, ethylamine, propylamine and *n*-butylamine were all effective in blocking clustering, and all showed a similar concentration dependence. *t*-Butylamine was slightly less effective than these amines in blocking clustering. Chloroquine (0.1 mM), lidocaine (1 mM), Tris-HCl (30 mM), *N*-acetyl-L-lysine-*N*′-methylamide (10 mM) and 1,4-diaminobutane-2HCl (putrescine) (10 mM) did not block clustering. It is apparently necessary for the amines to be inside the cells for the clustering to be inhibited, as simultaneous addition of methylamine and R-α_2M resulted in extensive clustering. The failure of putrescine to block clustering is probably due to its low rate of uptake[9], compared to aliphatic monoamines, which diffuse through the membrane as the uncharged species.

The fluorescence intensity of cells incubated with R-α_2M in the presence or absence of methylamine was approximately the same, indicating that the binding of α_2M by surface receptors is not blocked by the amine. Furthermore, R-α_2M which was bound diffusely to the cells in the presence of methylamine appeared in a punctate distribution 20 min after removing the amine from the medium (Fig. 1*c*). Fluorescence photobleaching recovery measurements[4,10] (F.R.M., unpublished) demonstrated that amines did not reduce the mobility of α_2M–receptor complexes.

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The inhibition of clustering by methylamine was confirmed using immunocytochemical localisation. Figure 2*a* demonstrates the diffuse labelling of the plasma membrane. There was an occasional slight concentration of label in these conditions in a coated pit, but this degree of clustering is considerably less than that seen in the absence of methylamine (Fig. 2*b*). The morphology of the cells was not changed in any obvious way by methylamine treatment.

Removal of Ca^{2+} from the medium was also found to inhibit the clustering of R-α_2M (Fig. 1*d*). The R-α_2M bound to the cells in the absence of extracellular Ca^{2+} did not form clusters when fresh medium containing Ca^{2+} (without R-α_2M) was added to the cells. However, the cells were still able to form clusters after EGTA treatment; R-α_2M added with fresh Ca^{2+}-containing medium formed clusters normally. EGTA itself was not responsible for the inhibition of clustering. When excess (4 mM) Ca^{2+} was added to medium containing 1 mM EGTA, R-α_2M formed clusters on the cells. Magnesium was not able to substitute for calcium. Evidently, calcium must be present as the α_2M binds to its cell surface receptor, as addition of calcium after removal of the R-α_2M from the medium did not result in clustering.

EGF has previously been shown to form clusters which coincide with the clusters formed by α_2M[1,3]. Figure 3 shows that 30 mM methylamine blocks the clustering of a rhodamine-labelled derivative of EGF. As with α_2M, binding to cell surface receptors was not inhibited by methylamine.

It has been suggested that ammonia, chloroquine and lidocaine might block the degradation of EGF by the same mechanism, perhaps by inhibition of proteolysis in lysosomes[11]. Our results indicate that the proximal effect of ammonia (but not chloroquine or lidocaine) in blocking EGF degradation is the inhibition of clustering. Ammonia also blocks the degradation of human choriogonadotropin (HCG) by murine Leydig tumour cells, without affecting HCG-induced steroidogenesis[12]. We speculate that the internalisation of this hormone is blocked by ammonia.

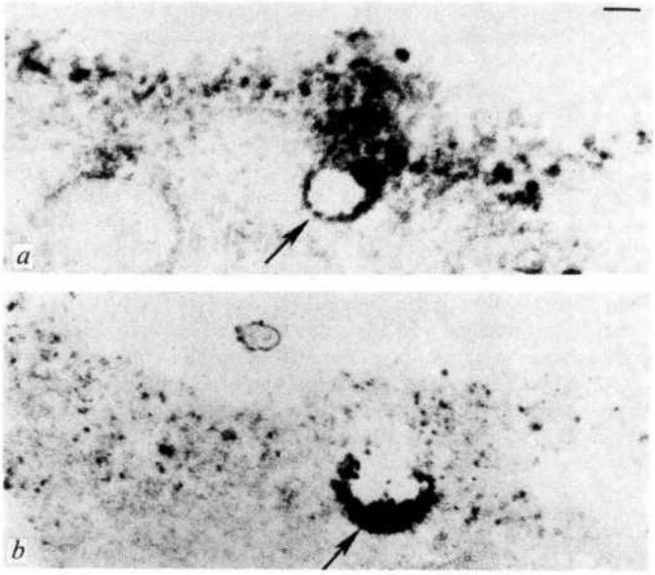

Fig. 2 Localisation of surface-bound α_2M. Cells were pretreated with 30 mM methylamine (*a*) for 20 min at 37 °C followed by incubation with 250 μg ml^{-1} α_2M for 5 min. They were then rinsed and fixed with 2% glutaraldehyde, and the surface-bound α_2M was detected using affinity-purified antibody to α_2M and peroxidase-conjugated anti-IgG (Cappel). Cells were incubated with diaminobenzidine substrate, post-fixed in 1.5% OsO_4, dehydrated in ethanol and embedded in Epon 812 as previously described[2]. Tangential thin sections were viewed without staining at 50 kV with a Hitachi HU-12A electron microscope. In *a* the diffuse labelling of the surface in 30 mM methylamine is shown, and a coated pit (arrows) shows little or no concentration of label above the rest of the surface. This is in marked contrast to the massive concentration of label in the coated pits in the absence of methylamine (*b*). Magnification, ×50,400; bar, 0.1 μm.

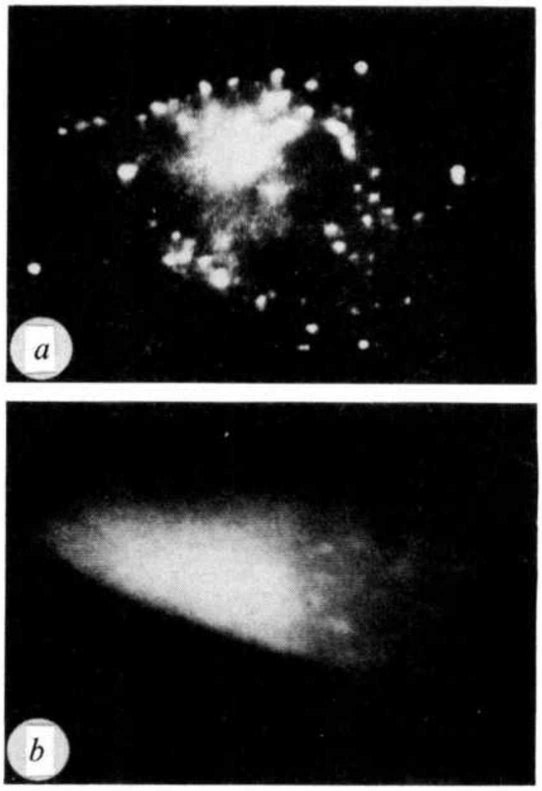

Fig. 3 Methylamine inhibition of EGF clustering. Cells were incubated in medium containing 1% fetal calf serum without (*a*) or with (*b*) 30 mM methylamine for 20 min at 37 °C. 50 ng ml^{-1} of rhodamine-labelled EGF was then added to the cells, and the cells were incubated for 20 min at 37 °C. They were then rinsed and fixed, and fluorescence was recorded as in Fig. 1. The rhodamine-labelled EGF consisted of rhodamine-labelled α-lactalbumin coupled to the α-amino group of EGF. The binding affinity of this derivative was shown to be the same as that of native EGF[16]. Magnification, ×1,130.

The mechanism for blocking clustering is not clear. One possible mechanism of action for the amines would involve an increase in the intracellular *p*H resulting from diffusion of uncharged amines across the plasma membrane followed by protonation inside the cell. It has been shown that chloroquine, methylamine and ammonia raise the lysosomal *p*H in this way[13]. The increase in *p*H could then, for example, interfere with a protein–protein interaction necessary for clustering.

An alternative is that the enzyme tranglutaminase is involved in the formation of clusters of receptor–ligand complexes over coated pits. This enzyme catalyses the formation of ε-(γ-glutamyl) lysine crosslinks between proteins[14]. It requires calcium for activity and is inhibited by ammonia and aliphatic amines, both of which are properties shared by the mechanism for the clustering of receptor–ligand complexes. ε-(γ-glutamyl) lysine crosslinks have been found in high concentrations in the plasma membrane proteins of mouse L cells[15]. According to this hypothesis, the binding of a ligand to its receptor would activate endogenous transglutaminase (through Ca^{2+} binding to the enzyme), which would then crosslink the receptor (or an associated protein) into the coated pit.

The inhibition of clustering by amines should prove useful in understanding the role of clustering and internalisation in various cellular functions. Hormone actions which require internalisation should be blocked by amines, and actions which occur only as a result of surface binding should be unaffected or enhanced. Further studies of the amine inhibition of clustering should help to elucidate the molecular mechanism for the clustering of ligand–receptor complexes.

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Peptide map analyses of murine Ia antigens of the I–E subregion using HPLC

THE I REGION of the major histocompatibility complex (H-2) of the mouse[1] controls various immunologically related functions including immune responsiveness and T-cell, B-cell and macrophage interactions[2,3]. The only gene products of the I region which have been identified by immunochemical techniques are the Ia antigens which are encoded by loci mapping to the I-A and I-E subregions of the I region[4]. Previously there has been ambiguity over whether the I-E and/or I-C subregion(s) control molecules detected by immunoprecipitation. Recent evidence suggests that the former, not the latter, controls these molecules[5,6]. The Ia antigens are highly polymorphic by serological analysis and are expressed predominantly on B lymphocytes and epidermal cells[7]. Ia molecules are integral cell-surface glycoproteins and consist of two subunits of approximate molecular weights 35,000 (α) and 28,000 (β) which are noncovalently associated[7]. A general nomenclature has been agreed upon for the Ia polypeptides[8]. For example, the α polypeptide from the I–E subregion of a mouse of the H-2^d haplotype is denoted E^d_α. Partial N-terminal amino acid sequence analyses of Ia polypeptides from the I–E and I–A subregions of mice[8–14], humans[14,15] and guinea pigs[16] have led to several important observations. (1) The E_α polypeptides of mice show striking homology with human α chains (p34) at least at their N-termini. The E_β polypeptides of mice also demonstrate some homology with their guinea pig and human counterparts. (2) The Ia polypeptides from the I–A subregion of mouse show little apparent homology with their human, guinea pig and I–E-subregion counterparts. (3) The A_α, A_β and E_β polypeptides of various haplotypes differ by one or more residues at their N-termini. However, for the E^d_α and E^k_α polypeptides no amino acid differences have been unequivocally demonstrated. To determine whether the N-terminal differences in the E_β polypeptides extend throughout other regions of these polypeptides and whether the E_α polypeptides are identical, we have undertaken tryptic peptide analyses of two Ia molecules from the I–E subregion. We report here the application of high-pressure liquid chromatography (HPLC) to the separation of tryptic peptides and present comparative tryptic peptide maps for I–E subregion products from the H–2^d and H–2^k haplotypes.

Table 1 Genetic constitution of recipient–donor–target strain combinations of mice used to prepare alloantisera and Ia polypeptides

Strain		Haplotype	Regions of H-2 K	I A	 B	 J	 E	 C	 S	 D
A. TH	(Recipient)	t2	s	s	s	s	s	s	s	d
A. TL	Donor	t1	s	k	k	k	k	k	k	d
B10. HTT	Target	t3	s	s	s	s	k	k	k	d
(B10×HTT)F$_1$	(Recipient)	b/l	b	b	b	b	b	b	b	b/d
B10 A (5R)	Donor	i5	b	b	b	k	k	d	d	d
B10. D2	Target	d	d	d	d	d	d	d	d	d

Boxed regions indicate the specificity of the antiserum against the target.

The procedures used for isolation and separation of the tryptic peptides of Ia polypeptides are described in Fig. 1. Table 1 gives the genetic constitutions of the mice used in the preparation of the alloantisera and the Ia antigens. The specificities of the antisera have been discussed elsewhere[10,11]. Reverse-phase HPLC has been used to separate peptides of biological significance[17] and tryptic peptides of globular proteins[18]. We now report its application to the separation of radiolabelled tryptic peptides of integral membrane proteins. We have found that the number of radiolabelled peptides which we detect by HPLC and conventional ion exchange, for both transplantation antigens (unpublished results) and Ia polypeptides, are comparable.

If the N-terminal homology observed between the E_α and E_β polypeptides and human Ia polypeptides continues throughout their entire structures, then from amino acid composition data for human Ia p29 (β) and p34 (α) polypeptides[15], we can expect 23–26 tryptic peptides per molecule. Freed has reported ~12 tritiated arginine-containing peptides for the E^k_β polypeptide using conventional ion-exchange chromatography[19]. We detected about seven tritiated tyrosine-containing peptides and about 15 leucine-containing peptides for the E^k_β polypeptide (Fig. 1). Accordingly, we are monitoring approximately 30–65% (7/23–15/23) of the tryptic peptides of the polypeptide.

Comparison of tryptic peptides of the E_β polypeptides shows that there are several common peptides and multiple peptide differences (Fig. 1). We conclude that multiple amino acid differences are present in several regions of the E_α polypeptides. In contrast, the E^k_α and E^d_α polypeptides do not show any definite peptide differences (Fig. 1). One major peptide of the leucine map of the E^d_α polypeptide seems to be broader than its E^k_α counterpart; we cannot explain this difference. As comparative peptide maps overemphasise amino acid differences and as we cannot detect any definitive peptide differences, we conclude that the E_β polypeptides must be extremely similar, if not identical.

These comparative peptide maps permit us to draw several interesting genetic and evolutionary conclusions about the Ia polypeptides.

(1) Homology relationships. Several Ia polypeptides of the I–A subregion have been analysed by HPLC of tryptic digests[12]. The E_α and A_α polypeptides share at most one tyrosine-containing peptide. The E_β and A_β polypeptides generally share only one or two peptides, although the A^k_β and E^k_β polypeptides show co-chromatography of four of seven tyrosine peptides. We do not know whether this apparent similarity is fortuitous or

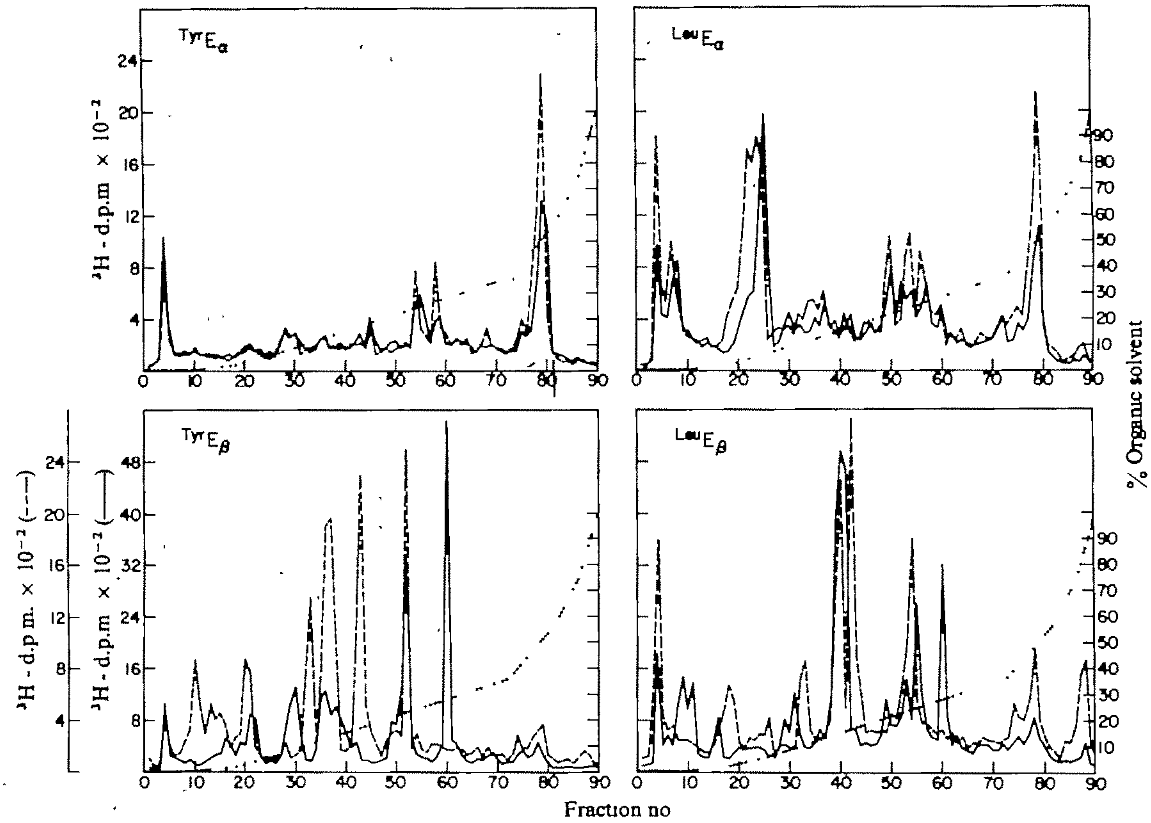

Fig. 1 HPLC tryptic peptide maps of α and β polypeptides of Ia molecules from the I–E subregion. ——, Peptides of the k haplotype, – – –, peptides of the d haplotype; · · ·, the concentration gradient of organic solvent used. Fraction number is plotted on the abscissa and the radioactivity of each fraction is plotted on the left ordinate. Ia antigens were biosynthetically radiolabelled and isolated by previously described procedures[10]. Splenic lymphocytes were cultured in the presence of either 1 mCi of tritiated tyrosine or 1 mCi of tritiated leucine. Radiolabelled Ia antigens were isolated by immunoprecipitation using specific alloantisera and *Staphylococcus aureus* Cowan I (ref. 23). The α and β polypeptides were separated by SDS–polyacrylamide electrophoresis[24] using 10% gels run in reducing conditions. The proteins were eluted from the gels in 0.01% SDS mixed with 0.5 mg porcine immunoglobulin and lyophilised. The polypeptides were reduced, alkylated and digested by the method of Brown[25] modified as follows. The polypeptides were boiled for 5 min in 0.5 ml of 0.6 M Tris, 0.02 M EDTA, 9% SDS, pH 8.3 buffer and reduced under argon for 3 h at 37 °C with dithiothreitol (0.025 M). The proteins were then alkylated in the dark for 1 h at room temperature using iodoacetamide (0.05 M) and reagents were then removed by centrifugation through a 2.5-ml column of Sephadex G-25 equilibrated with 0.05 M Tris, 2% 2-mercaptoethanol, 2% SDS, pH 7.5 (ref. 26). The proteins were then precipitated with 20% trichloroacetic acid, washed, dried and digested for 18 h at room temperature in 0.2 ml 0.05 M ammonium bicarbonate using 10 µl of trypsin–L(tosylamide-2-phenyl)-ethyl-chloromethyl-ketone (Worthington) (10 mg ml^{-1} in 0.001 M HCl). Another 10 µl of trypsin solution was added and after 4 h the resulting peptides were lyophilised. The peptides were analysed on a DuPont 830 HPL chromatograph using a 25-cm Zorbax-CN column at 49 °C. The column was initially equilibrated with 0.1 M sodium phosphate buffer, pH 2.1, and the peptides were eluted with a gradient of acetone (Burdick and Jackson). 1-ml fractions were collected every minute directly into scintillation vials and evaporated to dryness. 0.5 ml 0.01% SDS and 4 ml Aquasol-2 (NEN) were added to each vial and the radioactivity was then determined on a Beckman LS9000 scintillation counter

whether it signifies sequence homologies. In any case, it seems that the α and β polypeptides from the I–A and I–E subregions are not very closely related to one another.

(2) Genetic mapping. The mice from which the E^k and E^d molecules were isolated are congenic, presumably having distinct H-2 complexes superimposed on genetically identical B10 backgrounds. Haplotype-associated amino acid or peptide differences have been observed in the E_β polypeptides, which implies that the genes (structural or regulatory) controlling the synthesis of the E_β polypeptides are located within the H-2 complex. As the E_α gene product is apparently not polymorphic, no conclusions can be drawn about whether it lies in the H-2 complex. If the E^k_α and E^d_α polypeptides are indeed identical, the serological determinants on Ia molecules of the I-E subregion must reside on the corresponding E_β polypeptides.

(3) Complex allotypes. The E_β polypeptides exhibit multiple amino acid substitutions and can be defined as complex allotypes. The importance of distinguishing complex from simple allotypes, such as sickle-cell haemoglobin and its normal counterpart, is that a more complicated genetic organisation or evolutionary history is required to explain the generation of complex allotypes[26]. The α and β polypeptides of the I–A subregion and the K and D transplantation antigens, and the β polypeptide of the I–E subregion are all examples of complex allotypes. Thus, five distinct genes (or gene systems) encoded by the H-2 complex exhibit the phenotypic behaviour of complex allotypes.

Jones *et al.*[21,22] have reported that immunoprecipitates made using antisera having Ia specificities give a remarkably complex series of spots when analysed by two-dimensional gel electrophoresis and that these immunoprecipitates contain an invariant polypeptide of MW 31,000. We are now refining our peptide map procedures to identify which of these polypeptides have been analysed by amino acid sequence and peptide map techniques.

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Promotion of microtubule assembly *in vitro* by taxol

TAXOL (Fig. 1) was isolated from the plant *Taxus brevifolia* (western yew) by Wani *et al.*, who reported that the molecule has antitumour activity in several experimental systems[1]. In our laboratory we have found that taxol, a low molecular weight neutral compound, completely inhibits division of exponentially growing HeLa cells at low concentrations of drug (0.25 μM) that have no significant effects on DNA, RNA or protein synthesis during a 4-h incubation with the cells. HeLa cells incubated with taxol for 20 h are blocked in late G_2 and/or M (ref. 2). We report here that taxol acts as a promoter of calf brain microtubule assembly *in vitro*, in contrast to plant products such as colchicine and podophyllotoxin, which inhibit assembly. Taxol decreases the lag time for microtubule assembly and shifts the equilibrium for assembly in favour of the microtubule, thereby decreasing the critical concentration of tubulin required for assembly. Microtubules polymerised in the presence of taxol are resistant to depolymerisation by cold (4 °C) and $CaCl_2$ (4 mM).

Many models have been proposed for the microtubule assembly system used in these experiments[3], but the exact mechanism for microtubule assembly *in vitro* is not known; the conditions required have been described elsewhere[4]. A dynamic equilibrium of microtubules with tubulin dimers has been demonstrated *in vitro*[5–7]. Our standard conditions for assembly in a final volume of 1.0 ml at 37 °C are: 1 mM EGTA, 0.5 mM $MgCl_2$, 1 mM GTP, 0.1 M 2-[*N*-morpholino]ethane sulphonic acid (MES) at *p*H 6.6 and 1 mg ml^{-1} tubulin.

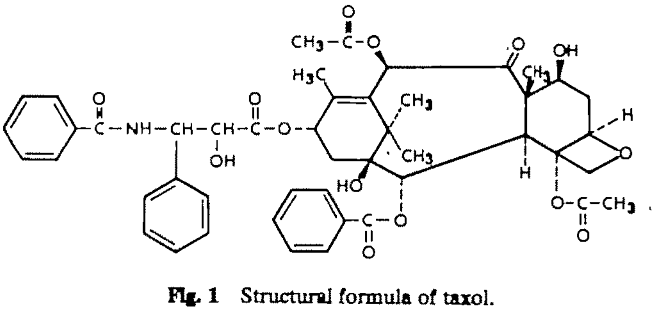

Fig. 1 Structural formula of taxol.

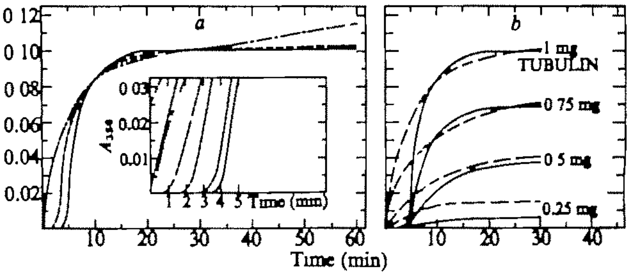

Fig. 2 Effect of taxol on microtubule assembly kinetics. Calf brain tubulin was prepared by two cycles of assembly–disassembly[19] and stored at −20 °C in MES buffer (0.1 M MES, 1 mM EGTA, 0.5 mM $MgCl_2$, adjusted to *p*H 6.6 with NaOH) containing 1 mM GTP and 4 M glycerol. Before each experiment the tubulin was dialysed for 3 h at 4 °C against 100 vols of MES buffer, and centrifuged at 120,000*g* for 20 min at 4 °C. Tubulin present in the supernatant was maintained in an ice bath. Tubulin purified by this method is 85% pure as determined on 3–27% SDS-polyacrylamide gradient reducing slab gels[20]. Assembly was monitored by the method of Gaskin *et al.*[5]. The cuvettes (1 cm path) containing MES buffer, drug and 1 mM GTP were kept at room temperature before addition of tubulin and shifting to 37 °C. Turbidity measurements were made at 350 nm every 20 s on a Gilford spectrophotometer equipped with an automatic recorder and a thermostatically regulated sample chamber. Taxol was dissolved in dimethyl sulphoxide at 10 mM and stored at −20 °C. The final concentration of dimethyl sulphoxide (0.5%) had no detectable effect on the assembly of tubulin. *a*, Standard assembly conditions: no additions (——); 0.05 (–· · ·–); 0.1 (–––); 0.5 (–– ·); 1 (· · ·); 5 (–·–); and 10 (–· · · ·–) μM taxol. *b*, Standard assembly conditions: 0.25, 0.5, 0.75 or 1 mg ml^{-1} tubulin, 37 °C. No additions (——); 5 μM taxol (–·–).

Microtubule assembly was monitored by the increase in turbidity[5] and by electron microscopy. In our conditions, taxol causes a dose-dependent decrease in the lag time for assembly (Fig. 2*a*); whereas the control has a 3.5 min lag time, 5 μM taxol essentially eliminates the lag time. The control assembly progress curve is sigmoid, but addition of 5 μM taxol gives hyperbolic kinetics. Ribbon structures and dimers are seen with the electron microscope at 30 s with 1 mg ml^{-1} tubulin and 5 μM taxol; in the control there are only rings and dimers (data not shown). The ribbon structures observed at 30 s with taxol are probably intermediates in assembly, as only microtubules are seen at 30 min. Figure 2*b* demonstrates that taxol decreases the lag time independently of tubulin concentration.

Microtubules polymerised in the presence of 10 μM taxol are shorter than in the control. Average microtubule lengths were determined by electron microscopy[7] at 30 min, a time at which equilibrium was apparently reached. The average length of the control microtubules is 4.12 ± 2.12 μm, whereas the microtubules polymerised in the presence of 10 μm taxol have an average length of 1.49 ± 0.65 μm.

Microtubules were polymerised in the absence and presence of 5 μM taxol for 30 min at 37 °C, and then chilled in an ice bath for 30 min. By electron microscopy, microtubules were seen only in the drug-treated sample. Ribbon structures, but no microtubules, were observed when polymerisation was carried out at 4 °C for 30 min in the presence of 50 μM taxol. Microtubules assembled at 37 °C in the presence of 10 μM taxol were completely resistant and those assembled in the presence of 5 μM taxol were partially resistant to depolymerisation by 4 mM $CaCl_2$ (Fig. 3*a*). Dimers, rings and microtubules were present 30 min after the addition of $CaCl_2$ to microtubules assembled in the presence of 5 μM taxol. Figure 3*b* demonstrates that microtubule assembly will take place in the presence of 4 mM $CaCl_2$ and 5 μM taxol.

Microtubules were centrifuged at 120,000*g* for 30 min and resuspended in 1 ml of MES buffer with or without 10 μM taxol. After 4 min, 4 mM $CaCl_2$ was added to the resuspended samples. Continuous turbidity measurements and electron microscopy indicated that no microtubules were present in the non drug-treated sample after the addition of $CaCl_2$, whereas

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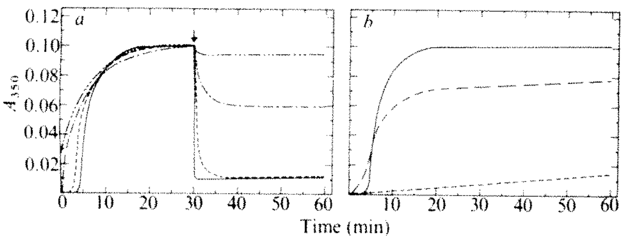

Fig. 3 Effect of $CaCl_2$ on microtubules assembled *in vitro* in the presence of taxol. *a*, Standard assembly conditions. No additions (——); 0.1 (– – –); 5 (– · –); and 10 (– · · · –) μM taxol. $CaCl_2$ is added at a final concentration of 4 mM at 30 min (↓). *b*, Assembly conditions as in (*a*). No additions (——); 4 mM $CaCl_2$ plus 5 μM taxol (– · –); 4 mM $CaCl_2$ (– – –).

the taxol-treated microtubules were resistant to depolymerisation by $CaCl_2$. Such experiments suggest that a taxol binding site is available on the intact microtubule.

Tubulin (7.7 μM; 1 mg ml^{-1} total protein) was assembled in standard conditions in the absence and presence of 10 μM taxol for 20 min. After centrifugation at 120,000*g* for 30 min, the amount of protein in the pellets and supernatants was determined[8]. In the absence of taxol, 0.57 mg of protein was pelleted by centrifugation. When tubulin was assembled in the presence of 10 μM taxol, 0.78 mg of protein was pelleted. A maximum amount of protein was pelleted at a taxol concentration approximately stoichiometric with the dimer concentration.

There is evidence that microtubule assembly *in vitro* takes place by the nucleated condensation mechanism of Oosawa and Kasai[9]. This model is supported by a requirement for a critical concentration (C_c) of protein to achieve assembly, and by tubulin–microtubule equilibrium data[5,6,10]. The critical concentration is approximately the inverse of the apparent microtubule propagation equilibrium constant (K_p^{app}) for the addition of a single tubulin dimer to a pre-existing microtubule[5].

$$\text{tubulin dimer} + \text{microtubule (length } n) \overset{K_p^{app}}{\rightleftharpoons}$$

$$\text{microtubule (length } n+1) \qquad \text{let } K_p^{app} = C_c^{-1}$$

Below the critical concentration, no microtubules are found by light scattering, electron microscopy or sedimentation velocity[5,10,11].

Taxol causes a decrease in the critical concentration of protein required for microtubule assembly *in vitro* (Fig. 4). Protein concentration was determined in both the supernatants and pellets. In the absence of drug (Fig. 4*a*), the critical concentration observed is 0.2 mg ml^{-1}(1.8 μM), a value which corresponds well with that previously reported[5]. In the presence of 5 μM taxol (Fig. 4*b*) the critical concentration decreased to ≤0.01 mg ml^{-1}(0.08 μM). The values for the apparent standard free energy (ΔG°_{app}) of addition of a tubulin dimer to a growing microtubule have been calculated using the formula $\Delta G^\circ_{app} = -RT \ln K_p^{app}$. For control and taxol-treated (5 μM) preparations, respectively, values for K_p^{app} were 5.7×10^5 and $\geq 1.1 \times 10^7$ l mol^{-1}, and for ΔG°_{app}, −8.0 and ≤ −10.0 kcal mol^{-1}. As taxol renders microtubules resistant to depolymerisation, the change in the apparent equilibrium constant could be the result of a reduced dissociation rate constant.

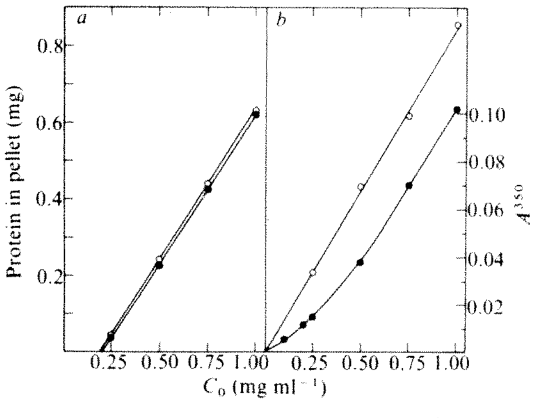

Fig. 4 Comparison of critical concentrations determined by centrifugation (120,000*g* at 25 °C for 30 min) of microtubules (○) and turbidity measurements (●) in the absence (*a*) and presence (*b*) of 5 μM taxol. C_0 is the total protein concentration. Determinations are made after a 30 min incubation in standard assembly conditions.

Turbidity at 30 min did not change appreciably compared with that of the control until the protein concentration was less than 0.25 mg ml^{-1} (Figs 2*b*, 4). Microtubules, tubulin hoops[12] with less than 13 protofilaments, and ribbons were observed at 0.1 mg ml^{-1} tubulin in the presence of 5 μM taxol at 30 min, whereas the control contained no structures (Fig. 5). At 1 mg ml^{-1} tubulin and 5 μM taxol, essentially no hoop structures or ribbons, only short microtubules, were found at 30 min.

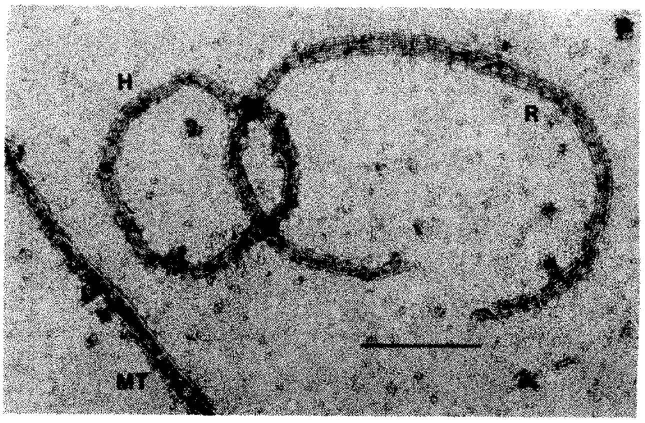

Fig. 5 Electronmicrograph of the structures assembled in the presence of 0.1 mg ml^{-1} tubulin and 5 μM taxol at 30 min in standard conditions. Structures were negatively stained with uranyl acetate[21]. MT, Microtubule; H, hoop; R, ribbon. Scale bar, 0.2 μm.

More and shorter microtubules are polymerised when assembly takes place in the presence of taxol. These shorter microtubules do not scatter light to the same degree as the control microtubules at the identical protein concentration[5]. However, as there are more of the short microtubules than there are of the control microtubules, there is very little difference in the amount of turbidity that develops at concentrations of protein above 0.25 mg ml^{-1} at 30 min. If the average length of the microtubules and the mass of protein pelleted after polymerisation in the presence of 10 μM taxol are taken into account, there is an approximate 3.8-fold increase in the number of microtubules (nucleations) in the drug-treated sample.

Phosphocellulose-purified tubulin, which is free of microtubule-associated proteins[13], will assemble in the presence of stoichiometric concentrations of taxol. Taxol also promotes assembly of charcoal-treated tubulin (1 mg ml^{-1}), which contains substoichiometric concentrations of GDP at the exchangeable site[14]. The drug has no effect on GTP or GDP hydrolysis in our microtubule preparations (data not shown).

There are similarities between microtubule and F-actin polymerisation. A nucleated condensation mechanism for the polymerisation of F-actin has previously been described[15]. Opposite-end assembly and disassembly have been shown for both F-actin polymerisation[16] and microtubule polymerisation at steady state *in vitro*[17]. Bound nucleoside triphosphate is hydrolysed during polymerisation in both systems. Phalloidin, a bicyclic peptide from the toxic mushroom *Amanita phalloides*, has been reported to accelerate the polymerisation of F-actin and confer resistance to depolymerisation[18]. Phalloidin and taxol represent natural products that enhance self-assembly systems. From our results, we consider that there may be low molecular weight cell constituents with activity similar to that of taxol which may be involved in the regulation of microtubule assembly in the cell.

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Molecular basis of thermostability in the lysozyme from bacteriophage T4

MOST proteins are denatured at temperatures above 50–60 °C, although some enzymes, especially those from thermophilic organisms, remain active at temperatures up to 80–90 °C. The determination of the three-dimensional structure of the thermostable protease thermolysin showed that heat-stable proteins do not contain unusual structural features absent from less stable proteins[1,2]. Furthermore, the amino acid sequences of similar proteins from both mesophilic and thermophilic sources have been shown to be homologous, suggesting that the respective structures are similar[3,4]. Nevertheless, such homologous amino acid sequences also include many differences which obscure those amino acid changes actually responsible for differences in thermostability. We report here the structure of a temperature sensitive (ts) mutant of T4 phage lysozyme. This permits the first direct comparison of two protein structures in which all differences are directly related to a change in thermal stability. It is shown that, except for the replacement of a partially exposed arginine by a histidine, the three-dimensional structure of the ts lysozyme is virtually identical with that of native lysozyme.

Bacteriophage T4 lysozyme, an endoacetylmuramidase, is produced late in the infection of *Escherichia coli* by T4 bacteriophage. It cleaves the bacterial cell wall, allowing the release of the progeny phage particles[5]. The amino acid sequence of the lysozyme has been determined[6], crystals suitable for detailed X-ray study have been obtained, the three-dimensional structure determined[7,8] to a resolution of 2.4 Å, and a thermodynamic analysis of the folding of the enzyme is underway (ref. 9 and unpublished results).

Since Streisinger developed a technique for identifying and isolating phage particles with mutations in the lysozyme gene[10], many different mutations in the lysozyme gene have been identified, the resultant changes in the amino acid sequence of the protein determined, and the properties of these mutant lysozymes discussed in the light of the three-dimensional structure of the wild-type enzyme[8,11].

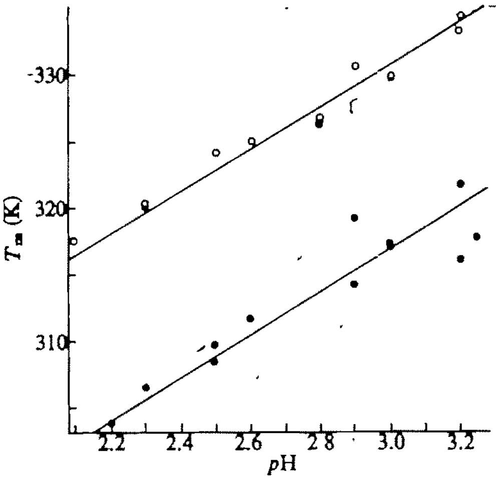

Fig. 1 The melting temperature (T_m) of wild-type (○) and temperature-sensitive (●) lysozymes as a function of *p*H. Denaturation was monitored by following changes in peptide circular dichroism at 223 nm. Details of the experimental method and of the analysis used to calculate T_m are given elsewhere[9]. Solvent conditions were as follows: 0.2 M NaCl, 10^{-4} M dithiothreitol, 10^{-6}–10^{-5} M lysozyme.

Lysozyme mutant strains were generated by use of 2-aminopurine, which induces single base pair substitutions. Temperature-sensitive mutants were selected applying the recognition method for lysozyme gene mutations[10]. Plaques of wild-type phage on plates incubated at 37 °C become surrounded by large haloes after exposing the plate to chloroform vapour. The haloes result from phage lysozyme diffusing from the plaque into the surrounding area and lysing the chloroform-treated bacteria. Halo size is a direct measure of the stability of the lysozyme. For ts mutants, the halo size decreases with increasing temperature on plates incubated at 30, 37 and 41 °C whereas wild-type plaque haloes stay large. By testing the ability of the mutant phage to recombine with a number of precisely mapped deletion mutants in the lysozyme gene, the locus of the amino acid change was determined to lie between residues 65 and 105.

The ts mutant lysozyme was purified according to the standard procedure for wild-type lysozyme[12], and was found to have a melting temperature about 14° lower than that of the native enzyme (Fig. 1). Crystals isomorphous with the native enzyme were obtained by the standard procedure[8]. The largest cell dimension change, in *c*, was 0.4 Å. We therefore were able to analyse the mutant by the standard difference Fourier technique (for example see ref. 13). A data set to 2.4 Å resolution was collected and a difference electron density map calculated between the native and the mutant lysozyme. Full details of the structure determination will be published elsewhere.

The difference density map is essentially featureless except at the location of the side chan of Arg 96, where a strong negative feature is seen at the site of the guanidinium group, and a positive peak overlaps the positions of C^{γ} and C^{δ} (Fig. 2). Clearly, Arg 96 in wild-type lysozyme has been replaced in the ts

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mutant by an amino acid with a medium-size side chain. The location of the substitution is consistent with that determined genetically. Determination of the amino acid composition of the mutant lysozyme revealed that there were two histidines, compared with one in the wild-type enzyme, indicating that Arg 96 has been replaced by a histidine. Furthermore, the electron density map (Fig. 2) also strongly suggests that the ts lysozyme has a histidine at position 96.

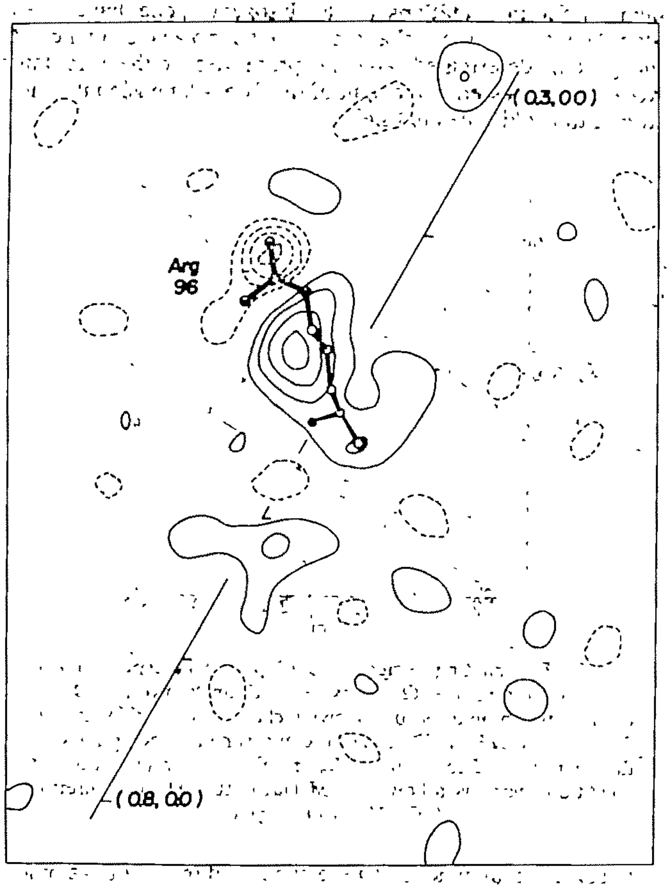

Fig. 2 Portion of the map showing the difference in electron density between native phage lysozyme and the temperature-sensitive mutant lysozyme with Arg 96 replaced by a histidine. The section shown, $z = 11/132$, approximately intersects the side chain of Arg 96. Contours are drawn at arbitrary equal intervals, with positive contours solid and negative broken. In the superimposed side chain of Arg 96, carbon atoms are represented by open circles and nitrogen by half solid circles.

In the native enzyme, the side chain of Arg 96 is well-ordered, and lies on the protein surface between the ring of Tyr 88 and the main chain and C^{β} of Leu 91 (Fig. 3). The hydrocarbon portion of the arginine side chain contributes to the major hydrophobic core of the C-terminal lobe of the molecule[7,8]. Substitution of an imidazole at this position presumably destabilises the hydrophobic core somewhat, providing at least part of the molecular basis for the observed temperature sensitivity. The imidazole ring of His 96 lies parallel to the ring of Tyr 88, and appears to donate a hydrogen bond to the carbonyl oxygen of the same residue (Fig. 3), possibly destabilising the α-helix 82–90. Detailed analysis of these interactions will take some time.

The main finding, however, which appears clearcut, is that the three-dimensional structure of the ts lysozyme is virtually identical with that of native lysozyme, except for the replacement of a partially exposed arginine by a histidine. Excluding residue 96, there is no evidence for structural changes larger than a few tenths of an angstrom. This finding is consistent with our earlier suggestion[2,14], based on the structure of the thermostable protease thermolysin, that differences in the thermostability of proteins are, in general, due to subtle changes in hydrophobic interactions, hydrogen bonds, and so on, and not to a single determinant such as metal binding or changes in secondary structure. In agreement with Perutz[15], our study shows that extra energy of thermal stabilisation can be provided without disturbance of the tertiary structure of the protein, although we do not see any change in the number of salt bridges in the mutant lysozyme. Also, our results confirm general thermodynamic arguments which suggest that the net free energy of stabilisation of proteins is small, and derives from a delicate balance between large stabilising forces, principally due to hydrophobic interactions, and large destabilising ones, principally due to chain entropy[16–18].

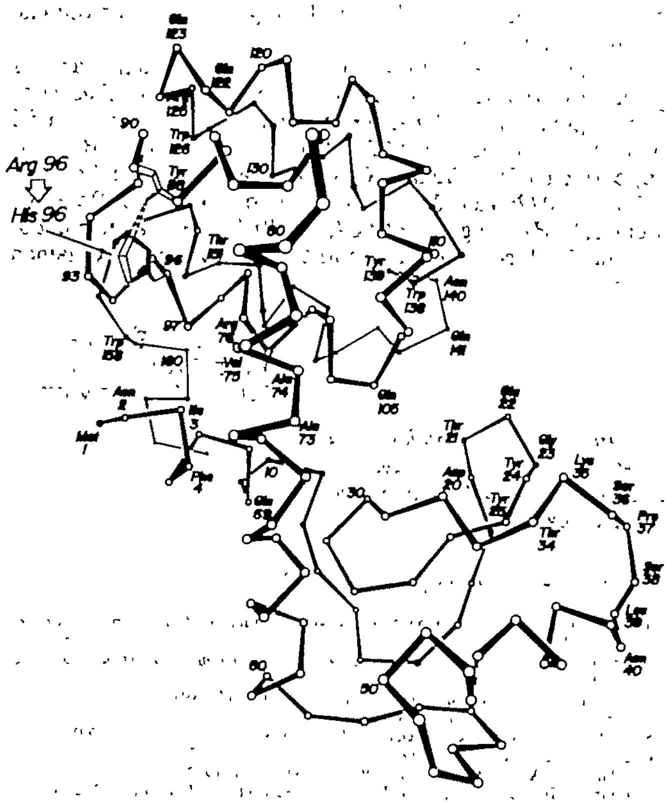

Fig. 3 Schematic drawing of the backbone of bacteriophage T4 lysozyme showing the location of His 96 in the temperature-sensitive mutant. (Adapted from ref. 8).

In addition to the mutant described here, three other temperature sensitive mutant lysozymes, with amino acid substitutions in other parts of the molecule, have been crystallised isomorphously with the wild-type enzyme. Clearly, these mutant lysozymes also have structures similar to that of the native enzyme. We hope that structural comparisons of these and other modified lysozymes will eventually provide a detailed accounting of the contributions of individual amino acid residues to the stability of a protein. Related studies of mutant lysozymes with modified activity should help clarify the mechanism of action of the enzyme.

We thank Kerrie Rine for technical assistance; Drs G. Streisinger and J. Owen for advice on lysozyme genetics, and Dr J. Schellman for discussions on lysozyme stability. M.G.G. was supported by the Swiss NSF. This work was supported in part by NIH grants GM 21967, GM 20066 and GM 20195.

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Effect of a local anaesthetic on hydrocarbon chain order in membranes

THE mode of action of anaesthetics has provoked considerable interest and speculation[1,2]. For tetrodotoxin[3], it is clear that direct interaction of the anaesthetic with the sodium channel blocks nerve conduction; in other cases changes in lipid bilayer structure, such as membrane expansion[2], fluidus expansion[1] or surface-charge modulation[4], have been invoked. Recently, a mechanism involving an increase in lipid bilayer thickness has been suggested from results obtained using black film techniques[5–7]. We have investigated the hydrocarbon chain length of a lecithin bilayer in the presence and absence of the anaesthetic benzyl alcohol[8,9] using high-field deuterium nuclear magnetic resonance (NMR) spectroscopy of specifically deuterated lipids[10–16], to determine the changes in bilayer structure that occur on addition of the anaesthetic alcohol. We report here that at concentrations used for local anaesthesia there is no change in membrane thickness. By comparison, cholesterol (at 0.3 mol fraction) causes an ~0.46 nm increase in membrane thickness. We therefore suggest that the amount of solvent (tetradecane) in black lipid membranes, and hence their thickness, may be influenced by the presence of benzyl alcohol and cholesterol[5].

Figure 1*a* shows the effect of benzyl alcohol on the deuterium quadrupole splitting of a dimyristoyl lecithin labelled as $^2H_2$ in the sixth position of the 2-chain. As the concentration of benzyl alcohol increases, the quadrupole splitting and therefore the order parameter of the labelled methylene segment[13] decreases. This suggests a decrease in order of the 2-chain; cholesterol causes an increase[10,11,15,16]. Determination of the change in order parameter at each segment in the hydrocarbon chain permits evaluation of the total change in hydrocarbon chain length[10,13,17]. We determined the quadrupole splittings, and the order parameters, for a range of dimyristoylphosphatidylcholines labelled (as C^2H_2 or C^2H_3) at one of positions C-2, C-3, C-4, C-6, C-8, C-10, C-12, or C-14, in the 2-chain, at a total lipid–anaesthetic mol ratio of 1:3. In Fig. 1*b* representative spectra illustrate the effect of benzyl alcohol on the deuterium quadrupole splittings of a series of ^2H-labelled phosphatidylcholines, and Fig. 1*c* shows the effects produced by benzyl alcohol and cholesterol on these splittings. Benzyl alcohol and cholesterol have opposite effects on the order of the phosphatidylcholine acyl chains. Benzyl alcohol (above the gel–liquid crystal phase transition temperature of the phospholipid) causes a decrease in hydrocarbon chain quadrupole splitting or order parameter; cholesterol (above the gel–liquid crystal phase transition temperature of the phospholipid) causes an increase in hydrocarbon chain quadrupole splitting, or order parameters. Qualitatively, therefore, benzyl alcohol decreases and cholesterol increases hydrocarbon chain order.

At anaesthetic concentration, however, there is no effect of benzyl alcohol on membrane thickness. Local anaesthetic concentrations of benzyl alcohol are 4 mM for rat phrenic nerve and 12 mM for frog sciatic nerve[9]. At 40 °C the partition coefficient of benzyl alcohol between multi-bilayers of dimyristoyl

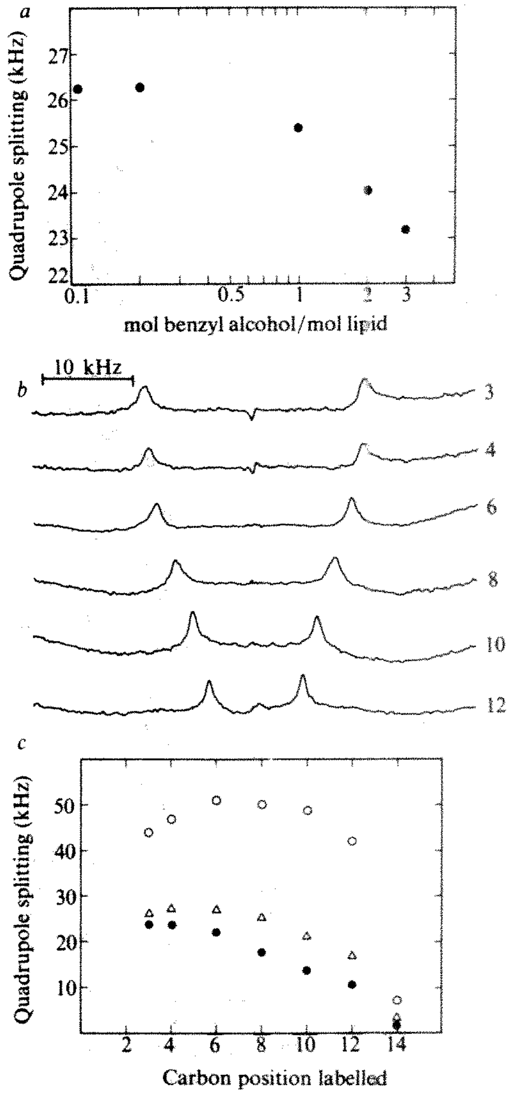

Fig. 1 Effects of benzyl alcohol, and of cholesterol, on deuterium NMR quadrupole splittings of specifically ^2H-labelled dimyristoylphosphatidylcholines. *a*, Graph of the quadrupole splitting of 2-(6′, 6′-d_2)dimyristoylphosphatidylcholine (10 mg) dispersed in excess deuterium-depleted water (250 μl) containing benzyl alcohol at 38° C, as a function of the benzyl alcohol–lipid mol ratio. The pure lipid quadrupole splitting value is 26.2±0.5 kHz. *b*, Representative deuterium Fourier transform NMR spectra of dimyristoylphosphatidylcholines, labelled in the 2-chain as C^2H_2 at the positions indicated. The samples contained a benzyl alcohol–lipid mol ratio of 3:1; spectra were recorded at 38 °C. *c*, Graph of quadrupole splittings of specifically deuterated dimyristoylphosphatidylcholines as a function of position labelled in the hydrocarbon chain, in the presence and absence of benzyl alcohol (3:1 benzyl alcohol–lipid mol ratio) or cholesterol (30 mol % cholesterol). Pure lipid and lipid–benzyl alcohol data obtained at 38 °C, lipid–cholesterol data obtained at 30 °C. Deuterium NMR spectra were obtained using the Fourier transform method at 5.2T (corresponding to a deuterium resonance frequency of 34 MHz) on a homebuilt instrument[10]. The synthesis of dimyristoylphosphatidylcholines specifically deuterated in the 2-chain are described elsewhere[10]. Benzyl alcohol was added as an aqueous solution to dry phospholipid and the mixtures homogenised at about 40 °C. △, Dimyristoylphosphatidylcholine alone; ○, in the presence of cholesterol; ●, in the presence of benzyl alcohol.

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lecithin and water is 13.9 according to Katz and Diamond[18]. At local anaesthetic levels, the benzyl alcohol–dimyristoyl lecithin mol ratio is 0.04–0.11. As may be seen from Fig. 1*a* there is no decrease in the electric quadrupole splitting from the pure lipid value of 26.2±0.5 kHz in this range of benzyl alcohol concentration, a result consistent with spin-label studies[9], although on a different timescale and without the possible complications of a perturbing probe effect.

At higher benzyl alcohol–dimyristoyl lecithin mol ratios there is a decrease in quadrupole splitting (or order parameter) with increasing concentration of benzyl alcohol, which agrees with spin-label studies[9] (Fig. 1). From these results it is relatively straightforward to determine quantitatively the projections of each C–C segment onto the bilayer normal[10,13,17], and so determine the effective length of the hydrocarbon chain, or the total membrane thickness[10,13,17,19]. Calculated chain lengths are rather insensitive to chain tilt. For example, for pure dimyristoylphosphatidylcholine at 60 °C, the distance from C-2 to C-14 is 10.2(6) Å assuming no chain tilt, or 10.5(2) Å assuming a gaussian distribution of chain orientations with a probable tilt angle of 25° (refs 10, 17). Similar average chain lengths are obtained using flat or lorentzian distribution functions (E.O. and R. Jacobs, unpublished). Consequently, we obtain from the NMR data a model-insensitive value for the average chain length, or membrane thickness. These lengths are in excellent agreement with those determined using high-resolution neutron diffraction (D. Worcester, M. Meadows, D. Rice and E.O., unpublished). For example, 23 °C lecithin–cholesterol data give a total hydrocarbon region thickness of about 30–31 Å, and neutron diffraction measurements[10] indicate a thickness of about 33 Å (Table III of ref. 10). From the data in Fig. 1*b* and *c* we can calculate the thickness of the dimyristoylphosphatidylcholine membrane at 38° C and obtain values of about 2.5(0) nm for the hydrocarbon region thickness in the absence of benzyl alcohol and about 2.3(6) nm in the presence of benzyl alcohol. The fluidisation of the bilayer caused by benzyl alcohol[8,9] decreases the membrane thickness. Cholesterol (30 mol%) causes an increase in hydrocarbon chain order, corresponding to about a 0.46-nm increase in membrane thickness at 23 °C (ref. 10).

Previous workers[5,6] have found, using black lipid membrane capacitance and conductance measurements, a 1.2-nm increase in membrane thickness in the presence of benzyl alcohol, and a decrease in hydrocarbon region thickness in the presence of cholesterol. Although the fatty acid composition of the lecithin used in the black lipid membrane studies differed slightly from ours, neutron beam and X-ray diffraction measurements on the egg-lecithin–cholesterol system indicate no significant decrease in membrane thickness on addition of cholesterol[21–23]. Similarly, other ^{2}H NMR and electron spin resonance spin-label studies of egg lecithin and egg-lecithin–cholesterol systems indicate an ordering of the hydrocarbon chains by cholesterol[15,24,25], which must correspond to an increase in projected chain length. In the absence of hydrocarbon solvents, such as tetradecane, an increase in projected chain length must correspond to an increase in membrane thickness, as neutron and X-ray data rule out $\geqslant$0.2 nm-chain interdigitation.

We therefore suggest that the previous black lipid film results[5,6] may be attributable to the inclusion of variable concentrations of solvent tetradecane in the lipid membrane, the exact amount being a function of the amount of cholesterol or benzyl alcohol present.

Deuterium nuclear magnetic resonance results suggest that the direct effect of benzyl alcohol on membrane bilayer thickness is negligible at the anaesthetic levels commonly used in clinical studies. At much higher levels (corresponding to an ~30–80-fold overload) there are small (≈0.1 nm) decreases in membrane thickness which are consistent with recent X-ray and neutron-beam studies of the effects of gaseous general anaesthetics on lipid bilayer thickness[26]. Taken together, our results cast considerable doubt on the importance of membrane thickness changes as a factor in the mechanism of action of many anaesthetics.

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X-ray diffraction patterns from molecular arrangements with 38-nm periodicities around muscle thin filaments

A SERIES of meridional and near-meridional reflections have been observed in X-ray diffraction patterns (ref. 1 and Fig. 1*a*) from the crab *Plagusia* leg muscle in the living, resting state, and have been attributed to troponin molecules which lie in pairs on thin filaments at 38-nm intervals. In the pattern (ref. 1 and Fig. 1*b*) obtained from the same muscle in rigor, the corresponding series of reflections was observed to have stronger intensities and higher order reflections. The reflections were interpreted as arising from both troponin and the cross-bridges. Electron micrograph[2] showed that cross-bridges occurred in symmetrical pairs around the thin filaments with a repeating distance of 38 nm in which one or a few closely spaced pairs may be included. Recently, reflections indexed as orders of 76 nm in X-ray diffraction patterns from insect flight muscle in rigor[3] and those from scallop striated muscle in rigor[4] have also been interpreted in terms of the cross-bridges attached in pairs to thin filaments with a repeating distance of 38 nm. We report here a method that enables us to analyse diffractions generated by molecular arrangements of this type. These arrangements are described by a set of identical helices, and the systematic modulation of intensities, indexed as orders of 76 nm, is interpreted in terms of displacement between these helices.

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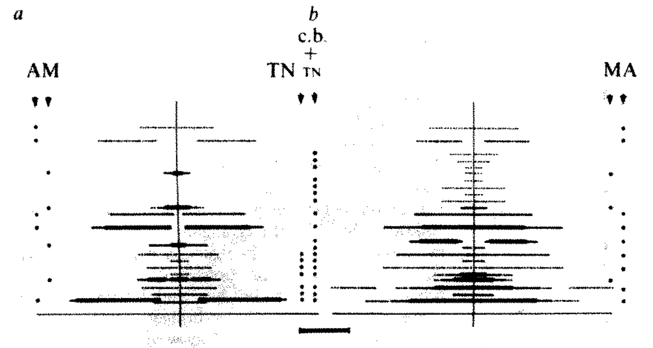

Fig. 1 Schematic representation of X-ray diffraction patterns[1] from crab *Plagusia* leg muscle in the living resting muscle (*a*), and in rigor (*b*). In addition to actin layer lines (marked by A) and meridional reflections (marked by M) attributed to myosin projections on the surface of the thick filaments, there is a series of meridional and near-meridional reflections (including intensities observed at $<0.07\ \text{nm}^{-1}$ on some of the actin layer lines) which were indexed as orders of 2×38 nm. In *a*, the reflections (marked by TN) were seen up to the 9th order, whereas in *b* those (marked by c.b. + TN) were observed up to the 24th. Thick solid lines, solid lines and dotted lines represent intensities observed even on the third film, up to the second one and on only the first film of each set, respectively. Bar, $0.1\ \text{nm}^{-1}$.

The thin filaments consist of actin, tropomyosin and troponin in a molar ratio of 7:1:1. The actin helix has a symmetry of 28/13 (28 molecules are included in 13 turns of the short-pitch helix) in the crab leg muscle in rigor. If we assume that these protein molecules interact with each other at specific sites, then the arrangement of troponin can be described by two identical 2/1 helices as shown in Fig. 2*a*. Axial displacement (Δz) and azimuthal rotation ($\Delta\phi$) between two molecules in pairs are specified by *k* multiple of those values between adjacent two-actin subunits along the 28/13 actin helix, where *k* is an odd integer. Ohtsuki[5,6] proposed a plausible model with $k = 1$, which remains to be proved.

Uniformly spaced layer lines, indexed as orders of 76 nm, are predicted from the single 2/1 helix as shown in Fig. 3*a*. Every even (odd) order Bessel function contributes to every even (odd) order layer line. The addition of another 2/1 helix results in a systematic intensity modulation which depends on *k*. As there is no trace of lattice sampling on the layer lines in the actual resting and rigor patterns from the crab leg muscle, we propose that the cylindrically averaged intensity[7] on each layer line can be written as

$$\langle F(R)F^*(R)\rangle_l = \sum_n J_n^2\,(2\pi Rr)|a_l^n|^2 \qquad (1a)$$

$$a_l^n = \sum_j \exp i\{-n\phi_j + 2\pi l z_j/c\} \qquad (1b)$$

where J_n is the *n*th order Bessel function, a_l^n the modulation term, and r, ϕ_j and z_j radial, azimuthal and axial coordinates of a molecule on the *j*th helix. In equation (1*a*), the effect of shape and size of a subunit on the intensity is not included. The summation in equation (1*a*) is taken over *n*, satisfying the selection rule[8] of $l = n + 2m$, where *m* is an integer. As $\Delta\phi = (1 - 1/14)\pi k$ and $\Delta z = c/28 \times k$, then $|a_l^n|^2 = 4\cos^2\{\pi k/28 \times (l+n)\}$ for l = even and $4\sin^2\{\pi k/28 \times (l+n)\}$ for l = odd. If $k = 1$ is assumed, even order Bessel terms would be dominant around the equator and $l = 28$, and odd orders would be dominant around $l = 13$ and 15 as shown in Fig. 3*c*, because modulation terms for meridional J_0 reflections (near-meridional $J_1 + J_{-1}$ reflections), for example, gradually vary with even (odd) values of *l*. If $k = 3$ is assumed, modulation terms vary three times faster with *l* (Fig. 3*d*).

In the actual resting pattern from the crab leg muscle (ref. 1 and Fig. 1*a*), (1) reflections are observed at every order from 2nd to 9th, except at 5th, (2) even order reflections have intensity maxima on the meridian whereas odd orders have not. These features are well explained only when $k = 1$. The presence of a meridional reflection at $l = 4$ and near-meridional one at $l = 9$ are hardly explained by $k = 3$. $k = 5$ and 7 are also excluded. The near meridional reflection at $l = 5$ may be buried in the strong reflection at 14.4 nm (assigned to thick filament). In the muscle in the resting state, the actin helix symmetry is slightly different from 28/13, and accordingly, the troponin helix is deformed slightly from 2/1. This difference is too small to affect the diffraction patterns[2]. We conclude that the troponin arrangement in Ohtsuki's model is valid for the thin filaments of the crab leg muscle, illustrating an application of this method in which intensity modulation is interpreted in terms of helical arrangement of molecules about the thin filament axis.

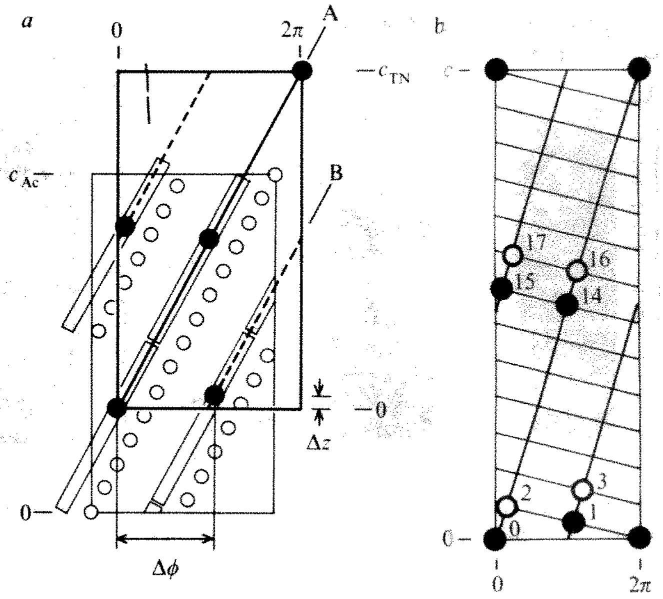

Fig. 2 *a*, The radial projection of the thin filament in Ohtsuki's model. A troponin molecule (closed circle) is attached to a specific site on a tropomyosin molecule (rectangular) which interacts specifically with seven actin subunits (open circles) arranged in a helix with the symmetry of 28/13 and the repeating distance of c_{Ac}. The arrangement of troponin molecules consists of two identical 2/1 helices, A and B, with the repeating distance of $c_{TN} = c_{Ac}$. Axial displacement (Δz) and azimuthal rotation ($\Delta\phi$) between two helices are specified by *k* multiples of those values between adjacent two actin subunits along the 28/13 actin helix, where *k* is an odd integer. Ohtsuki's model assumed $k = 1$. In the crab leg muscle[1], the symmetry of the actin helix is 28/13 in rigor, but in the living resting state it is between 26/12 (= 13/6) and 28/13. This means that the troponin helix differs slightly from 2/1 in the resting muscle. The solid frames represent one repeating unit of the structure. The shape of each molecule and the precise relationship between them are not shown. *b*, The radial projection of two possible models for the cross-bridge arrangement around the thin filament in crag leg muscle in rigor. When a pair of cross-bridges ($L = 1$) occurs symmetrically around the thin filament at intervals of 38 nm, four lattice points (closed circles) out of 28, labelled from 0 to 27 along the genetic helix, are occupied by cross-bridges. This arrangement is the same as the troponin arrangement shown in *a*($k = 1$). When two pairs ($L = 2$) of cross-bridges occur at positions adjacent to each other, four more sites (open circles) are occupied. In the latter case, the arrangement can be described by four identical 2/1 helices; each helix includes molecules labelled 0 and 14, 2 and 16, and so on.

If we assume that the head portions of myosin molecules are specifically attached to actin subunits, the surface lattice of cross-bridges around thin filaments in rigor (Fig. 2*b*) is the same as that of troponin molecules with $k = 1$, namely, cross-bridges occur in pairs at intervals of 38 nm. When another pair comes to the next position and both pairs are included at every 38-nm interval (Fig. 2*b*), the surface lattice can be described with four

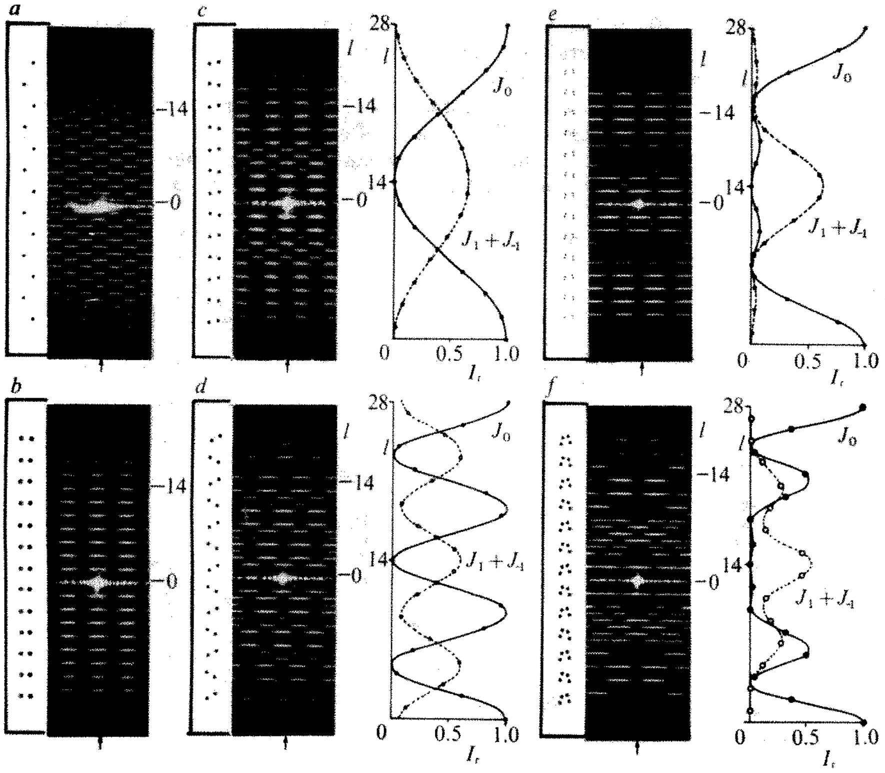

Fig. 3 Models of molecular arrangement around the thin filament and intensity modulations of diffraction patterns predicted from them. Models are *a*, single 2/1 helix; *b*, a pair of subunits in every 38 nm. The arrangement can be described with two identical helices with $\Delta z = 0$ and $\Delta\phi = 0$. *c*, The same as in Fig. 2*a*, *b* (closed circles only). The arrangement can be described with two identical 2/1 helices with $k = 1$. *d*, Two identical 2/1 helices with $k = 3$. *e*, The same as in Fig. 2*b* (closed circles plus open circles). *f*, Offer and Elliott model[10] for the arrangement of cross-bridges in insect flight muscle in rigor. In every 38 nm two pairs are attached to positions next but one to each other. In each set, the left is a model. Closed circles represent troponin and cross-bridges. The right of *a* and *b*, and the middle of *c*–*f* show optical diffraction patterns obtained from these models. To make higher order layer lines visible, the size of the circles in every model is chosen as that in *e*. The meridian is indicated by an arrow in each pattern. On the right in *c*–*f*, relative intensity maxima (I_r) of J_0 and J_1+J_{-1} contributions are plotted against l. These are calculated by equation (1), and the contribution of the form factor is neglected. In reality, unity in intensity in *e* and *f* is stronger by a factor of 4 than that in *c* and *d*. The necessary exposure time for the optical diffraction pattern in *a* is then 4 times as long as those in *b*, *c* and *d*, and 16 times as long as those in *e* and *f*. Note that if two molecules in pairs are axially aligned as in *b*, no odd order reflections can be observed. All the models are based on the actin helix of 28/13. Although the actin helix is slightly different in the crab leg muscle in the resting state, nevertheless, the difference is so small that the diffraction patterns are not affected[2].

identical 2/1 helices. When the number of pairs is increased to L, modulation terms, for example, for meridional J_0 reflections calculated by equation (1) are given as

$$|a_l^{n=0}|^2 = \sin^2(2L\pi l/28)/\sin^2(\pi l/28) \qquad (2)$$

which is an example of Laue function (Fig. 3*e*). Note that the more cross-bridges there are, the smaller the number of reflections on which intensities become concentrated. In the actual pattern from the crab leg muscle in rigor (ref. 1 and Fig. 1*b*), (1) meridional and near-meridional reflections were observed at orders from 2nd to 24th, of 76 nm; (2) meridional reflections at $l = 12$, 14 and 16 are absent, apparently due to a selection rule (if the form factor is diminished there, we cannot explain the fact that near-meridional components at $l = 13$ and 15 are observed in the actual pattern); (3) even order reflections have intensity maxima on the meridian, whereas odd order reflections have not. These features can be explained both by the model with $L = 1$ and by that with $L = 2$. In the diffraction pattern predicted from the $L = 2$ model, a few reflections (especially at $l = 2$) would be almost four times stronger and the rest would be weaker than in the $L = 1$ model. Nevertheless, we cannot decide between the two without measuring intensity distribution. Models with $L \geqq 3$ predict extinction of a meridional reflection at $l = 4$ in disagreement with the actual pattern. Any other model can be excluded. As we have pointed out[1], troponin molecules also contribute to these reflections. However, the result is not altered by the troponin contribution in this qualitative analysis of intensity modulation, as troponin does not make an important contribution to these intensities; the ratio of intensity of reflection in resting pattern to the corresponding one in the rigor pattern is smaller than 1/4 and higher order ($l > 9$) reflections are not observed in resting patterns.

To decide between the two possible models, and to determine the spatial relationship between troponin and cross-bridges on the same thin filament and also the size and shape of each molecule, we must make the analysis quantitative by obtaining a cylindrically symmetrical Patterson function[9].

Offer and Elliott[10] have proposed a model for the cross-bridge arrangement of insect flight muscle in rigor. In this model, two pairs of cross-bridges are included every 38 nm, but the second pair is positioned next but one to the first pair (Fig. 3*f*). The intensities predicted by the model, again based on intensity modulation calculated by equation (1), seem to be consistent with the recorded X-ray diffraction pattern[11]. In this muscle, the thin filaments are themselves arranged in a helical manner about the thick filament[12,13]. The absence of meridional reflections at $l = 2$ and 4 may be explained by the large helix. Lack of reflection at $l = 3$ might support the model, although other possibilities cannot be excluded. The disappearance of several near-meridional components of the diffraction pattern from the muscle after immersion in S-1 solution[3] could well be explained by the occurrence of more cross-bridges on the surface lattice with a symmetry of 28/13.

Vibert *et al.*[4] have recorded a series of reflections from scallop striated muscle in rigor which are thought to be similar to those from crab leg muscle in rigor. They observed an increase in intensities of near-meridional components at 38 nm and at 19 nm, after removal of the regulatory light chains. We might explain these observations by a rearrangement of the cross-bridges: from the arrangement with $L = 1$, for example, to that with $L = 2$. If this hypothesis is true, the intensities of some reflections would be decreased simultaneously.

I thank Professor F. Oosawa and Dr N. Sakabe for helpful comments on earlier drafts of this paper.

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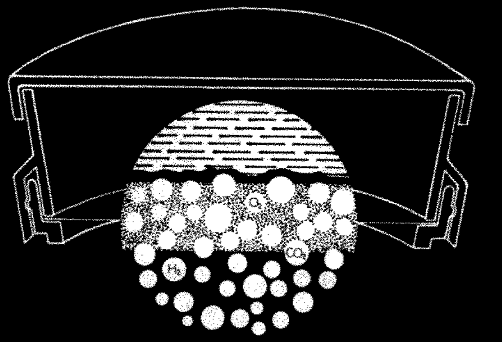

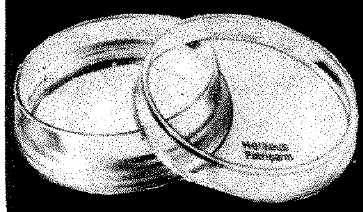

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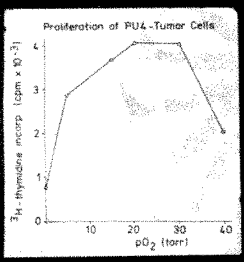

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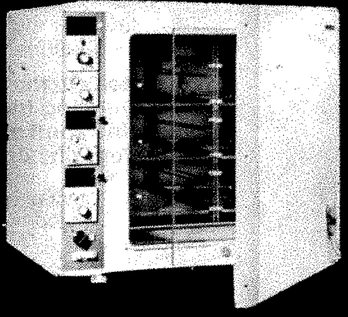

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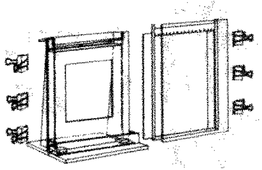

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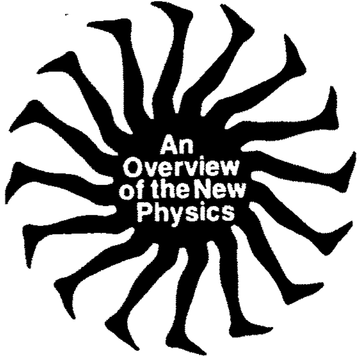
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reviews

Nature of chemical elements

G. N. Cantor

The Transcendental Part of Chemistry. By D. Knight. Pp. 289 (Dawson: Folkestone, UK, 1978.) £12.

CHEMISTRY, with all its associations with *praxis*, industry and sombre men in white coats, has or at least has had a 'transcendental' part as fascinating as that of any branch of physics. Yet the standard texts on the history of chemistry have all too often portrayed the development of their subject as an accumulation of experiments which lead directly to improvements in theory. By contrast this revisionist account, like some others of the genre, attempts to show both the subtle interplay between theory and experiment and the influence of metaphysical assumptions on both of these. In so doing Dr Knight and other recent historians of chemistry have uncovered some of the diverse intellectual roots of nineteenth-century chemistry.

This book concentrates on the theories of matter articulated by chemists and, in particular, their views about the nature of chemical elements. Two recurrent positions are identified: while some considered that the profusion of chemical substances were reducible to a very small number of fundamental types of particle—possibly even a single kind—though capable of many different arrangements, others adopted the view that each element was distinct and that it was the chemist's business to ascertain these, their properties and interactions. The relationship of these assumptions to practical chemistry is most clearly seen in the bitter dispute over the value of Prout's hypothesis. As Berzelius and others argued, experimental data refuted the hypothesis and thus seemed strongly to favour the latter position. However, the proponents of the former doctrine did not abandon the field but over a period of time made a strong comeback. The present book ends with Crooke's discovery of primary 'radiant matter' and, from the opposing party, Mendeleev's classification of the elements.

Much of the explicit history of chemistry discussed by Dr Knight is familiar terrain However, his interpretation of these events takes us far beyond the conventional bounds of chemistry. Matter theory is one of those areas influenced both by scientific and extra-scientific issues, and has thus provided a particularly interesting topic for historical analysis. For example, chemists of the early nineteenth century were able to draw on a stock of earlier theories such as those of Newton and his elaborators. Moreover, contemporary developments in many branches of science—such as electrochemistry, optics and heat theory—depended on and thereby affirmed certain current theories of matter. Finally, Dr Knight discusses the influences of theological, philosophical and other conceptual issues, citing evidence that some chemists conceived their matter theories as a bulwark against materialism and thus atheism.

The wide scope of this subject is both the book's strength and its weakness. The reader may at times lose the thread of the argument in moving rapidly from one topic to another and from the late eighteenth to the late nineteenth centuries. In very few cases do the specific relationships between metaphysics and practice emerge with the clarity which these complex issues deserve. Moreover, the philosophical underpinnings of matter theory—such as the definitions of 'matter', 'mechanism' and the role of analogy—deserve sustained analysis.

Despite these shortcomings Dr Knight has written a stimulating and enjoyable book which reflects the awareness among many historians that science has been influenced by extra-scientific factors and that a complex and intriguing relationship exists between metaphysical beliefs, scientific theory and experiment. ☐

G. N. Cantor is Lecturer in History and Philosophy of Science at the University of Leeds, UK.

Solid-state theory

Introduction to Solid State Theory. By O. Madelung. Pp. 486. (Springer: Berlin, Heidelberg and New York, 1978.) DM59; $29.50.

ONE might argue that solid-state physics is close to the point where the various parts are becoming so disparate and highly developed that there is no place for a relatively short (486 pages) graduate level account Professor Madelung's book on solid-state theory is intended for graduate students and research workers whose interests are primarily experimental, and provides a coverage of the main current theoretical ideas without using the formalism of modern many-body theory. He has produced a book which is small and coherent enough to be read in conjunction with a graduate course

The problem of writing a book of this sort is in the danger of either including discussions of a very wide range of subjects all of which are too brief to be useful; or concentrating on some aspects, and so giving an unbalanced account of the subject as a whole. The situation is complicated by the fact that there does not seem to be a consensus of opinion of what every solid-state experimenter should know. There are many obvious candidates such as, pseudo-potential theory, excitons, polarons, BCS theory of superconductivity, Boltzmann transport equation, the Hubbard Model, and so on. But equally there are many more subjects which may or may not deserve more than a passing mention; I would list here the Kondo effect, diffusion in ionic lattices, electron-hole recombination processes at imperfections, Anderson localisation (which Professor Madelung does include) and ferroelectricity and structural phase transitions, spin glasses, charge density waves, the Friedel sum rule and vitual bound states in metallic systems (which he does not). Everyone has his own list!

Pressing on with this argument one should ask: Is it a useful thing to try to do? Do we believe that an experimentalist concerned, say, with light scattering from magnetic insulators ought to know more than is present in the standard undergraduate textbooks on all, or any, of the topics I have mentioned? Also, how far is it useful to separate the theory of the behaviour of an isolated solid from the theory of the solid responding to some probe and if not which experiments should be included?

Professor Madelung believes that there are underlying themes in theoretical solid-state physics, and that this means that a study of one part of the subject is enriched by a knowledge of the rest. The primary theme that he has chosen is that of elementary excitations with a secondary theme of the dual descriptions of extended and localised states; and this has particular relevance to imperfect systems. The effects of uniform electric and magnetic fields have been included and most of the experiments which are discussed involve either absorption of light or simple transport properties.

The book uses the compact notation of second quantisation but calculations are performed using time-independent perturbation theory. This has the advantage that the new physical ideas are not obscured by unfamiliar formalism. There is a useful list of problems and topics for discussion to help the student assimilate the material. A further strength of the book is its excellent bibliography which includes a large number of review articles as well as books and original papers.

I find myself convinced that there is a place for a book of this length at this level and would recommend Professor Madelung's book, particularly to new graduate students.

Gillian Gehring

G. A. Gehring is the Physics Tutor at St Hugh's College, and a Lecturer in the Department of Theoretical Physics at the University of Oxford, UK.

Psychopharmacology handbook

Handbook of Psychopharmacology Edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. Vol. 7: Principles of Behavioral Pharmacology. Pp. 453. $41.40 Vol. 8: Drugs, Neurotransmitters, and Behavior. Pp. 590. $47.40. Vol. 9: Chemical Pathways in the Brain. Pp. 410. $35.40. (Plenum: New York and London, 1978.)

WITH these three volumes the Iversen–Snyder *Handbook* moves from the field of basic neuropharmacology into behavioural pharmacology. A total of 30 chapters contributed by 47 authors encompasses a range of subjects from the analysis of operant schedules (Dews and de Weese) to the localisation of gabaminergic neurones (Fonnum and Storm-Mathisen). The contributions are of a high standard and many are highly readable, but it is inevitable in an undertaking of this size that their relationship one to another is often uncertain Thus, each author attempts to produce a major review of his topic, but is bound to cover material which is also included elsewhere either in these or earlier volumes. Handbooks (and Handbücher) have always suffered from this problem, but of the genre the Iversen–Snyder production has much to commend it. In few other places can one find so much information relevant to the discipline.

Psychopharmacology is nothing if not cross-disciplinary. This should be most evident where two well established disciplines—for example, Psychology and Pharmacology—meet, as they do in volume 7 (Principles of Behavioral Pharmacology). It is interesting to see the extent to which one field has influenced the other In some cases the impact seems to have been small In the Dews and De Weese review referred to above drugs appear as another environmental variable to be accommodated by the same Skinnerian framework that describes other aspects of behaviour. The fact that such agents may be acting upon specific chemical mechanisms within the brain, and that these mechanisms may be regulating specific aspects of behaviour, is regarded as irrelevant to the description of drug action. The purely inductive approach seems, in this case, to act as a defence against asking some obvious, if difficult, questions. From a different psychological viewpoint Milner demonstrates a similar insularity. In giving a useful introductory survey to theories of motivation and learning which would be at home in a textbook of psychology, followed by some neural models, Milner implies that all this may be relevant to recent work on the physiology of central monoamine systems. Yet when it comes to making specific predictions from his own theory, or assessing the progress of other theories of the functions of monoamine neurones, Milner is reticent. It is at this point that the discipline of psychopharmacology (if there is one) gets interesting.

By contrast the work reviewed by Marshall and Teitelbaum (Neuropsychology of Motivated Behaviours) reveals the contribution which a rigorous application of the techniques of neuropsychology to preparations derived from advances in neuropharmacology (6-hydroxy-dopamine induced lesions of dopamine neurones) can make to our understanding of the mechanisms of behaviour. The theoretical implications of this work are considerable, though not developed at length by these authors, and give support to the view that neuroscience is in some respects leaving psychology behind. Other chapters in this volume cover major technical questions (for example, Robbins on motor activity, Mackintosh and others on ethological contributions and methods). Kumar has contributed a succinct but wide-ranging review of animal models of relevance to psychiatry, attempting to synthesise psychodynamic (for example, object loss), ethological, operant (for example, learned helplessness) and neuropharmacological, hypotheses. New dimensions to psychopharmacology are reviewed by Broadhurst (genetics) and Mabry and Campbell (development)

Many issues in volume 7 re-appear in volume 8 (Drugs, Neurotransmitters and Behavior), sometimes in a more controversial form. Here major motivated behaviours (eating, drinking, sleep) or motivational constructs (reward, punishment, fear, frustration) are foci for a discussion of pharmacological, behavioural and often many other aspects. Sometimes the relevance for psychopharmacology is obscure. For example, in a somewhat partisan account of the anatomy of self-stimulation, Routtenberg and Santos-Anderson make a virtue of discounting the neuropharmacological interest of the reward phenomenon. To this reviewer it remains an interesting and viable hypothesis that all reward

Applied group theory

Induced Representations in Crystals and Molecules: Point, Space and Non-rigid Molecule Groups. By S. L. Altmann. Pp. 369. (Academic: London, New York and San Francisco, 1978.) £18; $32 25. Review by H. C. Longuet-Higgins, *Nature*, **274**, 403 (1978).

DR ALTMANN has drawn the Editor's attention to certain sentences in Professor Longuet-Higgins' review, which he believes are open to criticism.

Professor Longuet-Higgins now writes:

First, my remark that Dr Altmann's groups are "virtually useless for classifying the rotational levels of non-rigid molecules" might have ben misconstrued to imply that the book deals explicitly with rotational levels. This it does not do, nor does it claim to do so, although Dr Altmann does give reference to theoretical work which is relevant to the treatment of rotational levels.

Secondly, the statement in the review that the symmetry operations relevant to an overall molecular state must "commute with the full molecular hamiltonian" could be taken to imply that the book defines symmetry operations to commute only with the Born–Oppenheimer hamiltonian; that is not the case.

Finally, my statement that "the book is, unfortunately, riddled with misprints . . . spelling mistakes ." could be read as suggesting that such errors were numerous. In fact I found very few.

I apologise unreservedly to Dr Altmann for having allowed these matters to escape my attention

H. C. Longuet-Higgins

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W. J. STEPHEN

This volume examines the provision of primary medical care in twenty-three different countries, selected because of their variety and importance. The underlying thesis of the account is that the health of any population depends more on the availability of good primary health care than on the advanced technology and sophistication of modern hospitals. The reasons why resources have been allocated in a way that does not reflect this view are argued fully. Note is also taken of the fact that medicine and politics have become interwoven in most countries of the world. **£12.50 net**

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T. W. GAMELIN

These lecture notes are devoted to an area of current research interest that bridges functional analysis and function theory. The topics covered include the rudiments of Choquet theory, various classes of representing measures, the duality between abstract subharmonic functions and Jensen measures, applications to problems of approximation of plurisubharmonic functions of several complex variables, and Cole's theory of estimates for conjugate functions. Many of the results, including Cole's theory, are published here for the first time.

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This book presents the scientific foundations of the mechanical behaviour of ceramics and demonstrates how these can be used in engineering situations. The emphasis is on principles, illustrated by a careful selection of experimental data. The treatment is at a level suitable for undergraduate and graduate students on materials-oriented courses and for research and development workers involved in engineering applications of ceramics.

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Edited by R. T. COUPLAND

This volume describes the structure and functioning of grassland ecosystems. Descriptions of the abundance and biomass of plants, animals and microorganisms are provided, as well as an assessment of the activities of each functional group of organisms in relation to production, consumption and decomposition. Static models of energy flow and nutrient cycling are included, and the effects on the ecosystem of the management of grazing land and cropland are also considered. This book is a companion to the forthcoming BP 19, *Grasslands, Systems Analysis and Man*, each representing an independent approach to the problems of studying grasslands.

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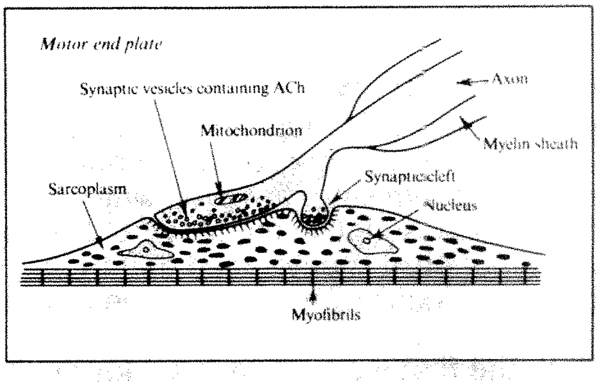

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R. W. RYALL

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The journal will provide a medium for the rapid publication of essentially short, definitive articles on marine organisms, which are broadly concerned with biophysical, biochemical and physiological processes as they affect the individual or the population.

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Marine Biology Letters will not be an appropriate medium for purely descriptive papers such as works on descriptive morphology, descriptive ecology or natural product chemistry. However, where the main purpose is to analyse or explain function, descriptive matter would not be excluded if it formed an integral part of the contribution. It is envisaged that the average paper will be in the region of 6-8 printed pages in length, but papers will not be rejected on account of brevity.

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signals are channelled through a final catecholamine element (whether dopaminergic or noradrenergic); whether or not this turns out to be the case it is the association of reward pathways with specific neurohumoural mechanism that must be of particular interest to the psychopharmacologist.

The balance is to some extent redressed by the discussion of Stein *et al.* of the neuropharmacology of reward and punishment. Noradrenaline features prominently, as expected, in relation to the former function, but dopamine now also has a place. The increasingly cogent evidence for a relationship between 5-hydroxytryptamine and punishment mechanisms, and their possible relevance to the actions of the benzodiazepines, is also reviewed. Diverse neuropharmacological approaches to feeding (Hoebel), drinking (Setler) and reproductive behaviour (Meyerson and Eliasson) are comprehensively handled, but many conceptual problems remain. In Jouvet's account of the current state of research on the neurohumoural mechanisms of sleep and waking it is apparent that much that earlier seemed to be clear on the basis of short-term experiments becomes obscure when the ability of the brain in the long term to compensate to apparently decisive insults is revealed.

This volume ends with provocative chapters by Gray on fear and frustration, and Hunter *et al.* on learning and memory. Gray believes that nonreward, punishment and novelty are classes of stimuli that have characteristics in common and may act by a single neurohumoural mechanism. His view that this includes the locus coeruleus projection to septum and hippocampus is in interesting contrast to the views of Stein and others expressed earlier in the volume. Hunter *et al.* review the evidence that noradrenaline and perhaps also 5-hydroxytryptamine and acetylcholine, may be directly involved in the acquisition and storage of new information.

In volume 9 the series returns from the knotty problems of correlating anything with behaviour to the more certain but rapidly expanding field of neurotransmitter pathway mapping. Here the juxtaposition of a number of reviews will be invaluable to the worker who wants a comprehensive account.

The *Handbook of Psychopharmacology* invites comparison with *Psychopharmacology: A Review of Progress 1957–1967* (ed. D. H. Efron, P.H.S. Publication 1836, US Government Printing Office, Washington, 1968) and its successor *Psychopharmacology: A Generation of Progress* (ed. M. A. Lipton, A. D. Di Mascio and K. K. Killam; Raven: New York, 1978). Both the *Handbook* and its competitors are good value. Reviews are presented in more detail, particularly with respect to techniques and their difficulties, in the *Handbook*. Therefore for a shorter review of an aspect with which he is unfamiliar, and particularly with viewpoints and theories, the reader may be advised to go the "Progress" volumes. For a more detailed account the *Handbook* presents much information in a readable form.

T. J. Crow

T. J. Crow is a member of the MRC staff and Head of the Division of Psychiatry at the Clinical Research Centre, Harrow, UK.

Metal ion solvation

Metal Ions in Solution. By J. Burgess. Pp. 481. (Wiley: New York, London and Toronto, 1978.) £25; $55.

As implied by the absence of the word "complex" from the title, this book is not concerned with metal complex ions in general. The author has wisely chosen to focus attention on the properties and certain reactions of metal ions that contain one or more solvent molecules as ligands. In view of the abundance of the present literature on inorganic solution chemistry, this is enough for any man to attempt. The result is a book that brings together in convenient form a wide variety of topics which are discussed in sufficient depth to provide a grasp of essentials and a stimulation to further study.

About two-thirds of the book deals with the study of metal ion solvation by spectroscopic and other techniques, thermodynamic aspects (thermochemistry and redox potentials), hydrolysis and polymerisation. The remaining third is concerned with kinetics and mechanisms—solvent exchange, anation reactions, redox reactions, and reactions of coordinated solvents. The solvolysis and stability constants of complexes are not covered. The treatment of aqueous systems necessarily forms a major part of the discussion, but there are frequent references throughout to non-aqueous solvents and a chapter on mixed solvents and selective solvation. Each topic is introduced at a level that requires a knowledge of basic physical chemistry and then progresses rapidly to the edge of the research literature.

The book is well organised, as shown by the extensive table of contents, and well documented with over 1,700 references and nearly 200 tables of

Vincent J. Maglio & H. B. S. Cooke
The Evolution of African Mammals
Current knowledge about the origin and evolution of the Class Mammalia in Africa is summarised in this book. The major geological deposits of the African Cenozoic are reviewed and the broader patterns of faunal evolution are identified. The modern mammalian fauna and environments are discussed, as is man's impact on wildlife. 77 line drawings, 66 halftones, bibliographies. February 1979, £42.00.

Geerat J. Vermeij
Biogeography and Adaptation
Patterns of Marine Life
Through this discussion of evolutionary process and adaptive response, Vermeij elucidates the general principles that underlie the great diversity of marine forms found in the world's oceans. 81 halftones, 9 line drawings. February 1979, £17.50.

B. Raymond Fink and Robert J. Demarest
Laryngeal Biomechanics
Fink and Demarest offer a new model of laryngeal function and provide both an atlas and a treatise of the behaviour of the larynx, for its size the most complex and versatile mechanical device in the body. Commonwealth Fund, 69 halftones, 26 line drawings. January 1979, £21.00.

H. J. Phaff, J. W. Miller & E. M. Mrak
The Life of Yeasts
2nd edition, revised & enlarged
The second edition of this book covers recent and major advances in knowledge of the morphology, physiology, genetics and ecology of yeasts, increasingly studied as prototypical eukaryotes. 15 line drawings, 13 halftones. January 1979, £10.50.

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data. The text is set out clearly and seems to be almost entirely free of misprints. There are a few criticisms, of course, but these arise mainly from personal prejudice as to the best way of teaching a particular topic and are perhaps most appropriately aired by direct communication with the author. According to the author the book is intended to occupy the middle ground between the elementary textbook and the erudite review. This is a fair claim. The enterprising undergraduate will find much of use and interest but, considering all the other parts of chemistry to be studied, will probably find the price beyond his means. Teachers will find the basis for courses at various levels in some important areas where inorganic and physical chemistry overlap. Research workers will find the necessary background (up to the end of 1975) to topics that border on their main fields of interest. There is something for everyone here. **J. S. Coe**

J. S. Coe is Senior Lecturer in the Department of Chemistry, King's College, University of London, UK.

Aspects of the nude mouse

The Nude Mouse in Experimental and Clinical Research. Edited by J. Fogh and B. C. Giovanella. Pp. 502. (Academic: New York and London, 1978.) $31; £20.15.

THE most provocative sentence in this interesting book is written by Rygaard. "If the nude mouse had appeared a decade earlier probably nobody would have paid any attention to the odd animal. Had it appeared a decade later most of the problems to which it may offer a solution would have been solved by other means". The last point is surely wrong, The congenitally athymic, nude mouse has been useful in confirming solutions that had been fairly well established using the less perfect T lymphocyte-deficient animals that immunologists have strived so hard to improve but the real value and fascination of this mutant arise from the new problems it has suggested. Thus the appearance of the *nu* gene would have stimulated a flurry of activity had it appeared at any time in the foreseeable future. Immunologists have achieved solutions to some of their problems but the strength of the subject lies in the number of soluble problems on our plate.

This is a timely, useful and well organised collection of chapters on many aspects of the nude mouse. The authors can be cleanly divided into those who regard the nude as a self-replenishing (although hopelessly complicated) test tube for sustaining human tumours, and those who exploit the nude to clarify the mechanisms by which normal and nude mice sometimes resist infection, grafts of foreign tissue and tumours. It is clear that the nude mouse reared under conventional conditions is near perfect in its incapacity to reject by an immune response grafts from other strains or from other species as distant as birds and reptiles. However nude mice do not in general show a higher incidence of either spontaneous or induced tumours. Rygaard and Povlsen found no tumours in a series of nudes observed for 5,600 years of mouse life. According to Stutman's thoughtful analysis the *nu* gene has probably no influence on the incidence of tumours: nudes seem to develop tumours less frequently than other mice because they are usually dead before they are six months old. Of all the observations in disagreement with the doctrine of immune surveillance this is the easiest to grasp. The T cell system of cell-mediated immunity is of established value in defence against infection and so it is unnecessary to search for additional advantages such as the infanticide of tumours.

If it is not done by T cells do animals use another system for stifling tumours and is this enhanced in the nude mouse? Here and elsewhere a great deal is written about NK cells—apparently lymphocytes possessing Fc receptors which kill a certain range of tumour cells *in vitro*. One is left convinced that the NK cell will come to be regarded as either the major immunological discovery of this decade or as a remarkable fiasco.

The ingenuity applied to the exploitation of the nude mouse is only just beginning. Two separate strains of nude, athymic rats have recently been discovered. These may or may not share precisely the same genetic defect with each other and with the nude mouse. In their turn they will confirm much of the new information gleaned from the nude mouse and will suggest fresh problems of their own.

W. L. Ford

W. L. Ford is Professor of Experimental Pathology at the University of Manchester, UK.

Catalytic processes

Chemistry of Catalytic Processes. By B. C. Gates, J. R. Katzer and G. C. A. Schuit. Pp. 464. (McGraw-Hill: New York, London and Toronto, 1978.) £21.35.

THIS is an interesting book which brings together the chemistry of catalysis and many of the concepts and techniques of importance to the operation of processes on the industrial scale. The plan adopted is to cover just five of the most significant types of catalytic processes and use them as a framework upon which to build. This approach might give the impression that the treatment of the subject would be more specialised and less general than is appropriate for a book of this kind, but, in fact, such an impression would be completely false. A great deal of fundamental chemistry is introduced as a basis for discussion of the chosen processes

The chapter on cracking provides an account of catalysis on amorphous and crystalline acidic surfaces, describes the chemistry associated with carbonium ion intermediates and in addition it covers aspects of reactor design needed for catalyst regeneration to avoid loss of activity from coking The second chapter is concerned with a number of reactions on transition metal complexes falling mainly under the classification of homogeneous catalysis; the basic chemistry included in this chapter deals with structure, bonding and reactions of organometallic complexes.

There is a very substantial amount of relevant fundamental material in the chapter on reforming. The treatment ranges from discussions of bonding in metals and alloys to consideration of the structures of typical oxide supports and from evidence for the nature of hydrogen adsorption on clean surfaces to the mechanisms of various hydrocarbon reactions on metals and on alumina.

The final chapters cover oxidation as exemplified by the ammoxidation of propylene and hydrodesulfurisation. Once again the same general combination of fundamental chemistry with relevant process engineering is provided in these chapters.

Taken as a whole this is a stimulating book which should appeal to a wide range of chemists and engineers who have a concern with reactions on surfaces. The novel approach and the skilful blend of recent ideas and developments with the more traditional aspects of the subject make the volume both attractive and timely.

Charles Kemball

Charles Kemball is Professor of Chemistry at the University of Edinburgh, Scotland.

Synopses of the British Fauna (New Series) edited by Doris M. Kermack and R. S. K. Barnes **No. 13**

British and Other Phoronids

C. C. Emig

March/April 1979, vi + 58pp., £2.80/$5.75 0.12.238750.3

Phoronids are a small, but widely distributed, group of benthic marine animals. The author provides keys and descriptions to enable not only the British species to be identified but also all other known species. This book, which is based on research over the last 20 years is the most recent review of the life history of phoronids and their morphological and anatomical characteristics. This synopsis includes full descriptions of species of phoronids occurring outside British waters.

The Soil-Root Interface

Proceedings of an International Symposium and published under the aegis of the New Phytologist

edited by J. L. Harley and R. Scott-Russell

March/April 1979, xx + 448pp., £15.40/$31.75 0.12.325550.3

While some aspects of the interactions between plant roots and the soil have been investigated for a century or more, there are others about which, very little was known until recently. The growth of interest in this field of research has to a large extent been due to the development of new experimental techniques which have permitted the examination of the more complex factors of water supply, nutrient availability and the pathology of plants. The specialized knowledge required for these studies necessitates a multi-disciplinary approach and those involved felt that there was a need to meet to discuss ideas and recent progress in the physical, chemical, biochemical, biological and microbiological characteristics of the interfacial region. This volume is the outcome of that meeting, which was held in Oxford in March 1978.

Studies of Language, Thought and Verbal Communication

edited by R. M. Blakar and R. Rommetveit

February/March 1979, approx. 500pp., £22.40/$47.25 0.12.594660.0

This book covers nearly two decades of theoretical and empirical inquiries into language, thought and human communication. A wide range of substantial issues is covered in the volume: from micro-analysis of the process of reading to patterns of communication in families containing schizophrenic members; and from contractual aspects of communication to analysis of language as a means of social power. Eight papers in Section 1 examine dominant traditions within research on language, thought and communication and outline an alternative conceptual framework. Section II follows with twenty-seven papers giving empirical support to this alternative social-cognitive framework.

An Introduction to Quarks and Partons

F. E. Close

March/April 1979, xiv + 472pp., £26.40/$54.50 0.12.175150.3

High Energy Physics has experienced a renaissance during the last four years, and the significance of quarks has become universally recognised. Since the excitement generated by recent discoveries such as that of the psi particle in 1974, and subsequently of 'charm', there has been no major *book* — as opposed to summer school reports — to cover progress in the rapidly developing field of High Energy Physics. This graduate text aims to correct this situation by presenting the basic ideas on quarks and partons at a level that can be understood by theorists and experimentalists with some familiarity with elementary particle physics or, simply, quantum mechanics. After this grounding, and with the aid of the comprehensive bibliography, the reader should be able to investigate the subject to whatever depth he chooses.

Annual Reports on NMR Spectroscopy Volume 8

edited by G. A. Webb

March/April 1979, x + 380pp., £28.00/$58.00 0.12.505308.8

The major aim of this widely-acclaimed series is to provide timely accounts of those areas of NMR spectroscopy where significant advances are being made. In recent years the advent of FT—NMR techniques has shifted the emphasis away from proton NMR towards less receptive nuclei such as 13c and 15N which are now being studied routinely in many laboratories. This, and recent progress in the theoretical interpretation of NMR parameters, is reflected in this series which covers NMR applications in all branches of science.

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The **JOURNAL OF NEUROSCIENCE METHODS** publishes research papers and a limited number of broad and critical reviews dealing with new methods or significant developments of recognised methods used to investigate the organisation and fine structure, biochemistry, histo- and cytochemistry, physiology, biophysics and pharmacology of: receptors, neurones, synapses and glial cells, in the nervous system of man, vertebrates and invertebrates. These studies may also be applicable to the clinical and behavioural sciences, tissue culture, neurocommunications, biocybernetics or computer software.

Although articles should be written in sufficient detail to allow others to verify these methods, they should also be intelligible to a broad scientific audience.
In addition, the journal publishes letters sent in camera-ready format, on paper supplied by the editorial office, containing comments or discussion of methodology described in this journal or any other journal devoted to the neurosciences.

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The following is a selection of papers appearing in initial issues. (First issue due March 1979)
A critical review of immunocytochemical methods for light microscopy *F. Vandesande (Ghent, Belgium)*
Techniques for precision beveling of relatively large micropipettes *K. T. Brown and D. G. Flaming San Francisco, Calif., U.S.A.)*
Photoelectrode with a very short time-constant for recording intracerebrally Ca^{2+} transients at a cellular level *E. Labeyrie and Y. Koechlin (Paris, France)*
Stereotaxic electrode placement in the neonate rat *A. Heller, J. G. Hutchens, M. L. Kirby, F. P. Karapas and C. Fernandez (Chicago, Ill., U.S.A.)*
Computer-assisted mapping with the light microscope *D. J. Forbes and R. W. Petry (Duluth, Minn., U.S.A.)*
A new moving-coil microelectrode puller *D. R. Ensor (Edinburgh, Scotland)*
Immunoglobulin elusion from multiple-sclerosis brain *D. H. Gilden and T. Tachovsky (Philadelphia, Pa., U.S.A.)*
A method of reversible inactivation of small regions of brain tissue. *J. G. Malpeli and P. H. Schiller (Champagne, Il., U.S.A. and Cambridge, Mass., U.S.A.)*

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announcements

Awards

The Royal Society has recently awarded the following medals: The Copley Medal to **Professor R. B. Woodward**; Royal Medals to **Professors R. A. Gregory, A. Salaam** and **T. Kilburn**; The Davy Medal to **Professor A. Eschenmoser**; The Darwin Medal to **Dr G. Pontecorvo**; The Leverhulme Medal to **Sir Frederick Warner**; The Mullard Medal to **Dr J. W. Black**; The Esso Medal to **A. G. Frame, J. M. Laithwaite, A. D. Evans, Dr J. F. W. Bishop** and **Dr T. N. Marsham**; The Rumford Medal to **Sir George Porter**; The Hughes Medal to **Professor W. Cochran.**

The following awards of the New York Academy of Science were made recently: The Distinguished Service Award of The New York Academy of Sciences was presented posthumously to **Margaret Mead,** eminent anthropologist and humanist, in recognition of her outstanding contributions to science and society; **Harrison Brown** has received The New York Academy of Sciences Award; **Anna Goldfeder** has received the Academy's Presidential Award; **Philip Leder** has received The New York Academy of Sciences Award in Biological and Medical Sciences. **Michael E. Fisher** has received The New York Academy of Sciences Award in Physical and Mathematical Sciences; **Michael Cole** has received The New York Academy of Sciences Award in Behavioral Sciences; **Peter Rentzepis** has received the A. Cressy Morrison Award in the Natural Sciences; **John J. McKetta** has received The Boris Pregel Award for Applied Science and Technology; **Paul Edward Garber** has received the I. B. Laskowitz Award for Research in Aerospace Engineering Sciences, Support Systems and Components; **Barbara McClintock** has received the Louis and Bert Freedman Foundation Award for Research in Biochemistry; **Victor Sidel** has received the Sarah L. Poiley Memorial Award; **Patrick Eggena** has received the Dr Harold Lamport Award; **Orville L. Chapman** has received the Gregory and Freda Halpern Award in Photochemistry.

Professor J. H. Burn, Emeritus Professor of Pharmacology, Oxford University, has been awarded the first Wellcome Gold Medal of the British Pharmacological Society.

The 1979 Israel Prize in the Life Sciences has been awarded to **Professor Hans Lindner,** Head of the Weizmann Institute's Hormone Research Department, for his outstanding achievements and most important contributions to endocrinology and the biology of reproduction.

The American Geophysical Union (AGU) has presented **Hsieh W. Shen,** Professor of Civil Engineering, Colorado State University, with the Robert E. Horton Award.

The first Wellcome Trust Award for Research in Biochemistry Related to Medicine has been made to **Dr D. J. H. Brock,** Reader in Human Genetics in the University of Edinburgh.

R. W. Whorlow, Senior Lecturer in the University of Surrey's Department of Physics, has received the first Annual Award of the British Society of Rheology, in recognition of his outstanding services to the Society for his work in the teaching and science of rheology.

Appointments

The President of the Royal Society, the Rt. Hon. the Lord Todd, OM, has appointed the following Vice-Presidents for the year ending 30 November 1979: **Dr B. J. Mason, CB,** Treasurer of the Royal Society; director-general of the Meteorological Office; **Professor D. C. Phillips,** Biological Secretary of the Royal Society; professor of molecular biophysics at the University of Oxford; **Dr T. M. Sugden, CBE,** Physical Secretary of the Royal Society; master of Trinity Hall, Cambridge; **Dr M. G. P. Stoker, CBE,** Foreign Secretary of the Royal Society; director of the Imperial Cancer Research Fund Laboratories; **Professor P. Allen,** professor and head of the department of geology and director of the sedimentology research laboratory in the University of Reading; **Sir Arnold Hall,** chairman and managing director, Hawker Siddeley Group Limited; **Professor D. C. Smith,** Melville Wills professor of botany and director of biological sciences in the University of Bristol; **Sir Michael Woodruff,** emeritus professor of surgery in the University of Edinburgh.

Professor Dennis J. Greenland has been appointed Deputy Director-General of the International Rice Research Institute in the Philippines.

Tony Benn, Secretary of State for Energy, has appointed **W. P. Blair** to be a member of the Advisory Council on Energy Conservation. Mr Blair is an Executive Council member of the Electrical, Electronic, Telecommunications and Plumbing Union (EETPU).

Dr Page Faulk, formerly Director of the Division of Developmental Immunology to The Medical University of South Carolina, US, has been appointed as Director of The Blond McIndoe Centre with effect from the 1st January 1979 and the Royal College of Surgeons of England have conferred on him the title of Honorary Research Professor.

Dr Charles M. Brown, senior lecturer in Biological Sciences at the University of Dundee, has been appointed to a new Chair in the Department of Brewing and Biological Sciences at Heriot-Watt University, Edinburgh. He will take up his appointment on 1 July 1979.

On 1 July 1979, **Dr Daniel A. Pollen** will become Director of Neurological Research and Chairman of the Division of Neurobiology at the Barrow Neurological Institute of St Joseph's Hospital and Medical Center in Phoenix, Arizona. Dr Pollen leaves the Neurosurgical Service of the Massachusetts General Hospital and Harvard Medical School where he was Associate Professor of Physiology in the Department of Surgery.

Dr J. W. Bridges, Reader in the University of Surrey's Department of Biochemistry and Director of the recently founded Institute of Industrial and Environmental Health and Safety, has been appointed to the new Chair in Toxicology—the only University Chair in Toxicology in the UK.

Joel L. Lebowitz, Professor of Mathematics and Physics and Director of the Center for Mathematical Sciences Research at Rutgers University, has been installed as president at The New York Academy of Sciences' 161st Annual Meeting held at The American Museum of Natural History on 5 December 1979.

Edward R. Hitchcock, a Birmingham medical graduate of 1952, has returned to the University as Professor and Head of the Department of Neurosurgery.

The Trustees of the Nuffield Foundation have approved a scheme devised by the Foundation's Committee on Rheumatism Research for the appointment of Senior Visiting Fellows in Rheumatism Research. The Committee hopes that the first appointment under this scheme will be made in time for the beginning of the academic year 1979–80. These Fellows will normally be based at a suitable laboratory in the UK during the tenure of their fellowship, but will be expected to visit most other centres of rheumatism research in Britain during the period of their appointment. Correspondence should be addressed to John Maddox, Director of the Nuffield Foundation, Nuffield Lodge, Regents Park, London NW1.

Meetings

2–6 April, **Appropriate Energy Technology for Development**, Glasgow (Dr J. Twidell, Appropriate Technology Seminar, Dept. of Applied Physics, University of Strathclyde, Glasgow, UK).

3–5 April, **Analytical Symposia**, London (T. Goodall, Pye Unicam Ltd, York Street, Cambridge, UK).

4–6 April, **Cell Adhesion and Motility**, Glasgow (Dr J. D. Pitts, Dept of Biochemistry, University of Glasgow, UK).

18–20 April, **ELSE, 4th General Assembly**, Budapest (Miss N. Morris, 30 Longdown Road, Lower Bourne, Farnham, Surrey, UK).

19–20 April, **Superconducting Electrical Machines**, Oxford (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

15 May, **Cancer—The Patient**, London (The Secretary, The Marie Curie Memorial Foundation, 124 Sloane Street, London SW1, UK).

16 May, **Industrial Applications of Particle Beams for Materials Analysis**, London (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

16–17 May, **32 Chemists' Conference**, Scarborough (K. Speight, British Steel Corporation, Sheffield Laboratories, Hoyle Street, Sheffield, UK).

2–13 July, **Factors Influencing Urban Design: A Systems Approach**, Louvain-la-Neuve (Dr P. Laconte, Expansion Dept., Université Catholique de Louvain, 13 Ave G. Lemaître, B-1348 Louvain la-Neuve, Belgium).

4–6 July, **194th Meeting of the Society for Experimental Biology**, Belfast (Dr M. W. Steer, Dept of Botany, Queen's University, Belfast, UK).

11–13 July, **Polypeptides Hormones**, Baltimore (E. G. Bassett, Miles Laboratories Inc., PO Box 40, Elkhart, Indiana, 46515).

15–22 July, **Bioethics and Public Policy**, Boulder (A. Perry, Institute of Society, Ethics and the Life Sciences, 360 Broadway, Hastings-on-Hudson, New York 10706).

16–19 July, **Endocrinology '79**, London (Conference Secretariat, The Endocrine Unit, Royal Postgraduate Medical School, Du Cane Road, London, W1, UK).

17–20 July, **INTERMAG—Magnetism and Magnetic Materials**, New York (Dr E. F. Luborsky, General Electric R&D Center, PO Box 8, Schenectady 12301).

19–21 July, **Genetic Aspects of Fertility and Fetal Wastage**, Southampton (European Society of Human Genetics, Dr M. Seabright, Wessex Regional Cytogenetics Unit, Salisbury General Hospital, Salisbury, UK).

1–3 August, **Theoretical and Practical Aspects of Recombinant DNA Techniques**, Sao Paulo (Prof. F. J. S. Lara, Dept. of Biochemistry, Institute of Chemistry, University of Sao Paulo and Institute Butantan, Sao Paulo 20780, Brazil).

13–24 August, **The Scientific and Mathematical Foundations of Engineering Acoustics**, Massachusetts (R. H. Lyon, Massachusetts Institute of Technology, Room 3-366, Dept. of Mechanical Engineering, Cambridge, Massachusetts 02139).

15–17 August, **20th Canadian High Polymer Forum**, Quebec (Dr. I. H. McEwan, Canadian Industries Ltd, Paint Research Laboratory, 1330 Castlefiled Avenue, Toronto, Ontario, Canada M6B 4B3).

26–31 August, **Biochemical Development of Nervous Tissue in Culture**, Tel Aviv (Dr E. Giacobini, Dept. of Biobehavioral Sciences, University of Connecticut, Storrs, Connecticut).

27–31 August, **The Teaching of Chemistry—Interaction between Secondary and Tertiary Levels**, Dublin (Professor D. J. Waddington, University of York, UK).

28 August–1 September, **Neurochemistry of the Retina**, Athens (Dr N. G. Bazan, Instituto de Invetigaciones Bioquimicas, Univ. Nacional del Sur—Conicet, Av. Alem 1253, 800—Bahía Blanca, Argentina).

Reports & Publications

UK & Ireland—January

Hebridean Naturalist, Vol 1, No 1/2, Summer/Winter 1978. Edited by I Stewart Angus. Pp. 1–84. (Stornaway, Isle of Lewis Western Isles Natural History Society, 5 Rathad Na Muilne, 1978.) £2.50 [15]

National Institute for Medical Research Report for 1977–78. Pp. iii+133. (London: Medical Research Council, 1978.) [16]

Health and Safety Executive Highly Flammable Materials on Construction Sites. Pp. iv+46 (London: HMSO, 1978.) £1 net. [17]

National Gallery Technical Bulletin, Vol. 2, 1978 Pp 76. (London: Publications Department, The National Gallery, 1978.) £2. [17]

Department of the Environment A Report on Captive Hawks. Pp. 10. (London: Department of the Environment, 2 Marsham Street, SW1, 1979.) [17]

Nuclear Power: Its Integration Into a Large Utility. By Glyn England. (Based on an Address to the British Nuclear Energy Society at the Institution of Civil Engineers, London, on 25 May 1978.) Pp 28. (London: Press and Publicity Office, Central Electricity Generating Board, 15 Newgate Street, EC1, 1978.) [18]

Health Service Commissioner First Report for Session 1978–79 Investigations completed—August to November 1978. Pp 254 (London: HMSO, 1978.) £3.50 net. [19]

Innovative Technologies for a Future Society. By Professor Carl-Goran Heden. Pp 24. (Scott Bader Commonwealth Monograph No 9.) (Wellingborough, Northants The Scott Bader Commonwealth, Ltd., Wollaston, 1979.) [22]

A Key to the British Species of Freshwater Triclads (Turbellaria, Paludicola) By T B Reynoldson. (Scientific Publication No 23.) Pp 31 (Far Sawrey, Ambleside, Cumbria Freshwater Biological Association, The Ferry House, 1978.) £1.30 [22]

Universities Research Reactor. Annual Report, 1977–1978. Pp iii+38. (Risley, Warrington. Universities Research Reactor, 1979.) [22]

Proceedings of the Royal Irish Academy Section A: Mathematical and Physical Sciences Vol 78, No. 17: On the Use of the Renormalization Group Near the Electron's Mass Shell By C Nash and R. L. Stuller. Pp. 149–156. £1.10 No. 18. The Use of Moutner Functions in the Identification of Heavily-Damped Systems. By J. C. L Dooge and B. J Garvey Pp. 157–179. £2.40 (Dublin. Royal Irish Academy, 1978.) [22]

Biotechnology Letters, Vol. 1, Part 1 Pp 1–59 Published monthly. Annual subscription Individuals £15 ($30), Libraries £50 ($100) (Kew, Surrey Science and Technology Letters, 12 Clarence Road, 1979.) [22]

Health and Safety Executive Health and Safety Manufacturing and Service Industries 1977. A Report of the Work of HM Factory Inspectorate incorporating the Annual Report of HM Inspector of Explosives. Pp. vii+84 (London HMSO, 1978.) £2.75 plus postage. [23]

Guide to Government Department and Other Libraries and Information Bureaux. Pp 92. (London: The British Library, Science Reference Library, 1978.) £3. [25]

Department of Health and Social Security Research and Development Report and Handbook 1978 (A Report and List of Projects Supported by the Department in the financial year 1977–78. Pp. v+167. (London. HMSO; 1978.) £4 net. [26]

Motorways and Agriculture: Severance. By Michael A. B Boddington, Alan S Hearne and P. M. K. Leat (Rural Planning Services Ltd. Publication No 5.) Pp. vi+33 (Homer, Ipsden, Oxon Rural Planning Services, Ltd., 1978.) £2 net. [26]

Bulk Refractories Industry: Energy Conservation and Utilisation in the Bulk Refractories Industry. (Energy Audit Series, No 4.) Pp 62. (London Library, Department of Energy, Thames House South, Millbank, 1978.) *gratis*. [26]

University College London Annual Report, 1977–1978 Pp. 137 (London: University College London, 1979.) [29]

International Vegetarian Health Food Handbook 1979–80. Pp. 208 (Altrincham, Cheshire: The Vegetarian Society, Parkdale, Dunham Road, 1979.) £1.20+15p postage [30]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 290, No 1373 A Precision Determination of the Lamb Shift in Hydrogen. By G Newton, D. A. Andrews, and P. J Unsworth. Pp 373–404. UK £2.10; Overseas £2.20. Vol. 290, No 1374. Resonance Tunnelling of Waves in a Stratified Cold Plasma By K. G. Budden, FRS Pp. 405–433. UK £1.90, Overseas £2 Vol. 290, No 1375. Discrete Symmetric Dynamical Systems at the Main Resonances with Applications to Axi-symmetric Galaxies. By F. Verhulst Pp 435–465 UK £2.05, Overseas £2.15 (London The Royal Society, 1979.) [31]

Other countries—January

Australia Commonwealth Scientific and Industrial Research Organization Annual Report of the Division of Textile Physics, 1977/1978. Pp 53. (Ryde CSIRO, 338 Blaxland Road, 1978.) [8]

Employment and Environment Pp. 68 (Paris: OECD, 2, rue André Pascal, 1978.) F18; £2.20, $4.50 [8]

Bulletin of the American Museum of Natural History. Vol. 161, Article 4: Revision of the *Myiarchus* Flycatchers of South America By Wesley E. Lanyon. Pp. 427–628. (New York. American Museum of Natural History, 1978.) $12.70. [8]

A Report to the President and Congress on the Status of Health Professions Personnel in the United States. (Hyattsville, Md US Department of Health, Education and Welfare, Public Health Service, Health Resources Administration, 3700 East-West Highway, 1978.) [9

US Department of the Interior: Geological Survey. Professional Paper 1064–A. Mixed-Layer Clay in the Pierre Shale and Equivalent Rocks, Northern Great Plains Region. By Leonard G. Schultz. Pp. iv+28 (Washington, DC: US Government Printing Office, 1978.) [9]

World Health Organization. International Classification of Diseases Ninth Revision. Basic Tabulation List with Alphabetical Index Pp 331. Geneva: WHO London: HMSO, 1978.) Sw.fr 15. [11]

Académie Royale de Belgique Mémoires de la Classe des Lettres. T. LXIII, Fasc. 3: Étude sur la Confédération Béotienne (447/6–386)—Son Organisation et son Administration. Par Pierre Salmon Pp. 269. (Bruxelles. Académie Royal de Belgique, 1978.) [12]

Energy, Mines and Resources Canada. Geological Survey of Canada. Bulletin 284. Lower Ordovician Acritarchs and Trilobites from Bell Island, Eastern Newfoundland. By W. T. Dean and F. Martin Pp. 35 (7 plates). (Ottawa: Geological Survey of Canada, 1978.) $3.50. [15]

Recent scientific and technical books

Applied biology

WARNER, Noel L., and COOPER, Max D. (edited by) Contemporary Topics in Immunobiology, Vol 8. Pp.xiv+272. ISBN-0-306-37808-6. (New York and London. Plenum Press, 1978.) $17.32.

WATKINS, J, and WARD, A. Milford Adverse Response to Intravenous Drugs. Pp.xiii+188. ISBN-0-12-737850-2 (London: Academic Press, New York Grune and Stratton, Inc., 1978) £8.20.

Psychology

BERKOWITZ, Leonard (edited by). Group Processes. (Papers from Advances in Experimental Social Psychology.) Pp.xiii+428. ISBN-0-12-091840-4 (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) $13.50; £8.75

BURGHARDT, Gordon M, and BEKOFF, Marc. The Development of Behavior: Comparative and Evolutionary Aspects (Garland Series in Ethology) Pp.xiii+429. ISBN-0-8240-7015-1. (New York and London Garland STPM Press, 1978.) $32.50.

FISHER, Seymour, and GREENBERG, Roger P. (edited by) The Scientific Evaluation of Freud's Theories and Therapy: a Book of Readings. Pp.xv+446 ISBN-0-85527-583-9. (Hassocks, Sussex: The Harvester Press, Ltd; New York: Basic Books, Inc., 1978.) £13.50.

FOSS, Brian M. (edited by). Psychology Survey, No. 1. Pp.221 ISBN-0-04-150066-0. (London, Boston, Mass. and Sydney: George Allen and Unwin, Ltd., 1978.) £2.50.

GOLDSTEIN, Arnold P. (edited by). Prescriptions for Child Mental Health and Education. (Pergamon General Psychology Series. Pergamon International Library of Science, Technology, Engineering and Social Studies) Pp ix+454 ISBN-0-08-022249-8. (Oxford and New York: Pergamon Press, 1978) Hard £11 25, Flexi £4.75.

HOWELLS, John D. (edited by) Modern Perspectives in the Psychiatric Aspects of Surgery. (Modern Perspectives in Psychiatry, Vol 7.) Pp x+756. ISBN-0-333-24370-6 (London and Basingstoke: The Macmillan Press, Ltd., 1978) £15 net

KRIPPNER, Stanley (edited by) Extrasensory Perception (Advances in Parapsychological Research, Vol 2) Pp.ix+308. ISBN-0-306-40082-0 (v.2). (New York and London Plenum Press, 1978) $14.17.

LOCK, Andrew (edited by). Action, Gesture and Symbol: The Emergence of Language. Pp.xiv+588. ISBN-0-12-454050-3. (London and New York. Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978) £22; $45.

MAHER, Brendan A. (edited by). Progress in Experimental Personality Research, Vol. 8. Pp.xiv+344. ISBN-0-12-541408-0. (New York and London Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978) $23.

MARKS, Lawrence E. The Unity of the Senses: Interrelations Among the Modalities. (Academic Press Series in Cognition and Perception) Pp.xi+289. ISBN-0-12472960-6. (New York and London. Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) $17.50; £11 35

MULLER-SCHWARTZE, Dietland (edited by). Evolution of Play Behavior. (Benchmark Papers in Animal Behavior/10.) Pp xiii+396. ISBN-0-87933-272-7. (Stroudsburg, Penn.: Dowden, Hutchinson and Ross., Inc, 1978. Distributed world wide by Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers.) $32.

PRINCE, Morton The Dissociation of a Personality. The Hunt for the Real Miss Beauchamp. Pp xxviii+575. ISBN-0-19-281249-1. (Oxford, New York and Melbourne: Oxford University Press, 1978.) First published in 1905.) £2 95 net.

RITCHIE, William C. (edited by). Second Language Acquisition Research: Issues and Implications. (Perspectives in Neurolinguistics and Psycholinguistics) Pp.xiii+221. ISBN-0-12-589550-X. (New York and London Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) $14.50; £9 40

SHERSHOW, John C. (edited by). Schizophrenia Science and Practice Pp.248. ISBN-0-674-79112-6. (Cambridge, Mass. and London. Harvard University Press, 1978) np.

STAUB, Ervin. Positive Social Behavior and Morality Social and Personal Influences, Vol. 1. Pp xvi+490. ISBN-0-12-663101-8. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978) $19 50, £12.65.

STELMACH, George E. (edited by). Information Processing in Motor Control and Learning. Pp xii+313. ISBN-0-12-665960-5. (New York and London Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978) $21; £13 65

STERNBACH, Richard A. (edited by) The Psychology of Pain. Pp x+271 ISBN-0-89004-278-0 (New York. Raven Press, 1978) $25.35.

TEYLER, Timothy (edited by). Brain and Learning. Pp 163 ISBN-90-90-277-0808-8. (Dordrecht: D. Reidel Publishing Company, 1978 Sold and distributed in North America by Greylock Publishers, 13 Spring Street, Stamford, Conn.) Dfl 50, $19 50.

THORNTON, Raymond (edited by) Smoking Behaviour Physiological and Psychological Influences Pp 405 ISBN-0-443-01815-4. (Edinburgh, London and New York. Churchill Livingstone, 1978) £15.

VYGOTSKY, L. S. Mind in Society: The Development of Higher Psychological Processes. Edited by Michael Cole, Vera John-Steiner, Sylvia Scribner and Ellen Souberman. Pp.xi+159. ISBN-0-674-57628-4. (Cambridge, Mass and London Harvard University Press, 1978) £8 75.

WASSERMAN, Gerald S Color Vision. An Historical Introduction. (Wiley Series in Behavior.) Pp.xv+224. ISBN-0-471-92128-9 (New York and Chichester Wiley-Interscience, John Wiley and Sons, 1978.) £13.40.

General

ASPECTS OF HUMAN SETTLEMENT PLANNING. Edited by Habitat Conference Secretariat. Pp. vii+288 ISBN-0-08-022011-8. (Oxford and New York: Pergamon Press, 1978.) £21.25.

BALABAN, Miriam (edited by). Scientific Information Transfer. The Editor's Role. (Proceedings of the First International Conference of Scientific Editors, April 24–29, 1977, Jerusalem) Pp.xxxiii+686 ISBN-90-277-0917-3 (Dordrecht, Boston, Mass. and London: D. Reidel Publishing Company, 1978.) Dfl 70.

BELLAMY, David. Botanic Man: a Journey Through Evolution with David Bellamy. Pp.208. ISBN-0-600-31457-X. (London and New York The Hamlyn Publishing Group, Ltd., 1978.) £6.50.

BOOTHROYD, Hylton. Articulate Intervention. (ORASA ext No. 1.) Pp.xii+154. ISBN-0-85066-171-4. (London. Taylor and Francis, Ltd, 1978. Published in association with the Operational Research Society) £6.

BORN, Max. My Life. Recollections of a Nobel Laureate Pp.308. ISBN-0-85066-174-9. (London: Taylor and Francis Ltd, 1978) £8 50 net.

BUKOVSKY, Vladimir. To Build a Castle: My Life as a Dissenter. Translated from the Russian by Michael Scammell. Pp.352. ISBN-0-233-97023-1. (London Andre Deutsch, 1978) £7.50 net

CAPLAN, Arthur L (edited by). The Sociobiology Debate: Readings on Ethical and Scientific Issues. Pp xiv+514 ISBN-0-06-010633-6. (New York and London: Harper and Row, Publishers, 1978.) £7.95.

CLARKE, David L. Analytical Archaeology. Second edition. Revised to Bob Chapman. Pp xxi+526. ISBN-0-416-85450-8. (London. Methuen and Co., Ltd., 1978.) Hardback £12.95 net; Paperback £7.95 net.

CROSLAND, Maurice. Gay-Lussac: Scientist and Bourgeois. Pp.xvi+333. ISBN-0-521-21979-5. (Cambridge, London and New York: Cambridge University Press, 1978) £15 net.

GABOR, D., COLOMBO, U., KING, A., and GALLI, R. Beyond the Age of Waste. (A Report to the Club of Rome) (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xvii−237. ISBN-0-08-021834-2. (Oxford and New York Pergamon Press, 1978.) £6.

GOODMAN, Leo A. Analyzing Qualitative/Categorical Data Log-Linear Models and Latent-Structure Analysis Edited by Jay Magidson. Pp.viii+471. ISBN-0-201-02505-1. (Reading, Mass. and London: Addison-Wesley Publishing Company, 1978.) $25.

GREENBERG, Michael R. Applied Linear Programming for the Socioeconomic and Environmental Sciences. (Operations Research and Industrial Engineering.) Pp.xv+327. ISBN-0-12-299650-X. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978) $19; £12.35.

HOY, Ken. On Nature's Trail: An Illustrated Introduction to the Sights and Signs of the Countryside. Pp.128. ISBN-0-86134-004-3. (London: Artists House, Mitchell Beazley Marketing, Ltd., 1978) £6.95.

JOLLY, Clifford J. (edited by). Early Hominids of Africa. Pp.x+598. ISBN-0-7156-0928-9. (London: Gerald Duckworth and Co., Ltd., 1978.) £28 net.

LANCASTER, F W. Toward Paperless Information Systems (Library and Information Science) Pp.xii+179 ISBN-0-12-436050-5 (New York and London. Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) $13.50, £8.75.

LARSEN, Thor. The World of the Polar Bear Pp.96 ISBN-0-600-37155-7. (London and New York: The Hamlyn Publishing Group, Ltd., 1978) £3 50.

LASZLO, Ervin The Inner Limits of Mankind. Heretical Reflections on Today's Values, Culture, and Politics. (Pergamon International Library of Science, Technology, Engineering and Social Studies Systems Science and World Order Library) Pp xiii+79. ISBN-0-08-023012-1. (Oxford and New York. Pergamon Press, 1978.) Hard £7; Flexi £3.50

LEBEDEV, N I. A New Stage in International Relations. Translated by D. Ya Skvirsky and V M. Schneerson. Pp xv+253 ISBN-0-08-022246-3. (Oxford and New York: Pergamon Press, 1978.) £10

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STARLING, Alfred (Bud). Enjoying Indiana Birds Pp xvii+308. ISBN-0-253-31956-0. (Bloomington and London: Indiana University Press, 1978.) $17.50

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Mathematics

BURKILL, J C A First Course in Mathematical Analysis Pp.vi+186 ISBN-0 521-29468-1 (Cambridge, London and New York: Cambridge University Press, 1978. First published 1962) £3.95

DUDLEY, Underwood. Elementary Number Theory. (A Series of Books in the Mathematical Sciences) Pp ix+249 ISBN-0-7167-0076-X. (San Francisco and Reading W H Freeman and Company, 1978.) £8.70 Second edition.

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FINKBEINER, II, Daniel T. Introduction to Matrices and Linear Transformations Third edition. (A Series of Books in the Mathematical Sciences.) Pp.xii+462, ISBN-0-7167-0084-0. (San Francisco and Reading: W. H Freeman and Company. 1978.) £10 30.

FRIEDMAN, Avner, and PINSKY, Mark (edited by) Stochastic Analysis. (Proceedings of the International Conference, April 10–14, 1978, Northwestern University, Evanston, Inn.) Pp.xii+340. ISBN-0-12-268380-3. (New York and London Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers. 1978) $18, £11 70

LOCKWOOD, E. H., and MACMILLAN R H. Geometric Symmetry. Pp.x+228 ISBN-0-521-21685-0. (Cambridge, London and New York: Cambridge University Press, 1978.) £10.50.

NIJENHUIS, Albert, and WILF, Herbert S. Combinatorial Algorithms for Computers and Calculators Second edition. (Computer Science and Applied Mathematics a Series of Monographs and Textbooks.) Pp.xiii+302. ISBN-0-12-519260-6. (New York and London Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978) $19.50; £12.65.

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SEHGAL, Sudarshan K. Topics in Group Rings. (Pure and Applied Mathematics. a Program of Monographs, Textbooks, and Lecture Notes.) Pp.vi+251. ISBN-0-8247-6755-1 (New York and Basel. Marcel Dekker, Inc, 1978) $24.50.

Astronomy

KAUFMANN, III, William J. Stars and Nebulas. Pp.ix+204. ISBN-0-7167-0081-6. (San Francisco and Reading. W. H Freeman and Company, 1978.) Hardcover £8.20, Softcover £4.10.

REDDISH, V. C. Stellar Formation (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xii+287. ISBN-0-08-023053-9. (Oxford and New York: Pergamon Press, 1978.) Hard £15 Flexi £7.50.

ASEARS, D. W. The Nature and Origin of Meteorites. (Monographs on Astronomical Subjects. 5.) Pp x+187 ISBN-0-85274-374-2. (Bristol: Adam Hilger, Ltd., ($78.) £13.50.

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Applications are invited from suitably qualified graduates for a Lectureship in Biochemistry in the Biochemistry Department. Preference will be given to applicants with postgraduate experience and an interest in mammalian or physiological biochemistry. Industrial experience would also be advantageous.

Salary will be within the range £3,883 to £7,754 per annum according to qualifications and experience.

Further particulars about the post may be obtained from the Academic Registrar (LFG), University of Surrey, Guildford, Surrey GU2 5XH, or Tel: Guildford 71281, Ext. 452. Application from men or women in the form of a curriculum vitae, together with the names and addresses of two referees, should be sent to the above by March 12, 1979. 9761(A)

CHAIRPERSON, PROFESSOR OF CHEMISTRY

DEPARTMENT OF CHEMISTRY

State University of New York at Albany—Applications are invited for this position to start on September 1, 1979 or as soon thereafter as possible. The Department of Chemistry has a commitment to continuing development of outstanding programs in research and teaching and seeks a Chairperson to provide leadership in these areas and to guide the future growth of the department. The applicant should be a chemist of senior rank, with an established reputation in research and interest in teaching and administration. Each applicant should send a resume and the names of at least three references to: J. A. Zubieta, Chairman, Search Committee, Department of Chemistry, State University of New York at Albany, Albany, New York 12222. Applications should be completed before March 30, 1979. An EO/AA employer. 9743(A)

Graduate Biologist

In our group studying new approaches to drug therapy of enteric disease a vacancy exists for a pharmacologist or physiologist with a particular interest in gastrointestinal function.

We should be pleased to consider newly qualified graduates in the above disciplines (2:i Hons. degree minimum standard) through to PhDs with up to one year's post doctoral experience. Experience of *in vivo* experimentation is desirable and an interest in goal orientated research and drug evaluation essential.

Walton Oaks is pleasantly situated in the Surrey countryside near Dorking and Reigate. The Company provides excellent working conditions including flexible working hours.

Application forms (to be returned by March 16) may be obtained from **Mrs. M. R. Minchin, Personnel Officer, Beecham Pharmaceuticals Research Division, Animal Health Research Centre, Walton Oaks, Dorking Road, Tadworth, Surrey. (Telephone: Tadworth 4444).** 9785(A)

Beecham Pharmaceuticals

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the post of

SENIOR LECTURER

in the

DEPARTMENT OF VETERINARY PATHOLOGY

Candidates must be veterinarians with a Ph.D. in Pathology and teaching experience at University level. Salary scale: K£2,988 to K£3,984 per annum (K£1 equals £1.34 sterling). The British Government may supplement salary by £5,430 per annum (sterling) for married appointee or £3,648 per annum (sterling) for single appointee (reviewed annually and normally free of tax) and provide children's education allowances and holiday visit passages. Family passages; superannuation scheme; medical aid scheme; various allowances. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent direct to Registrar, University of Nairobi, P.O. Box 30197, Nairobi, Kenya by April 5, 1979. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9720(A)

MEDICAL RESEARCH COUNCIL

LABORATORY ANIMALS CENTRE

TWO RESEARCH OFFICERS

One post is in the Centre's Accreditation Department. Its duties will include visiting commercial breeders of laboratory animals throughout Great Britain who are accredited or recognised by the Centre.

One post is in the Director's Department and is concerned with a research programme into Specific Pathogen Free cats, with some administrative responsibility for the day-to-day running of the department.

Candidates for either post should have at least two years' postgraduate experience in biology or zoology; a knowledge of small animals would be an advantage but not essential. Salary according to age, qualifications and experience plus £354 London Weighting. Contributory Superannuation Scheme.

Applications, together with a copy of curriculum vitae and the names of two professional referees, should be addressed to: The Director, M.R.C. Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF.

MRC
Medical Research Council
9621(A)

UNIVERSITY COLLEGE CARDIFF

Applications are invited from registered Medical, Dental or Scie
graduates for the post of

LECTURER

in the

DEPARTMENT OF ANATOMY

The appointment carries responsibil for teaching preclinical medical an or dental students at ordinary a honours levels. The appointee m engage in research work. Salary ran £3,883 to £7,754 per annum. Dut to commence not later than October 1979.

Applications (two copies), togetl with the names and addresses of thi referees, should be forwarded to t Vice-Principal (Administration) a Registrar, University College, P. Box 78, Cardiff CF1 1XL, from wh further details may be obtained. Cl ing date: March 31, 1979. Referer 1720. 9765(A)

UNIVERSITY OF NOTTINGHAM

SCHOOL OF AGRICULTURE

TEMPORARY LECTURESH in SOIL SCIENCE

There will be a vacancy for Lecturer in Soil Science in the Depa ment of Physiology and Environment Studies. The appointment will be f two years from September 1, 197 with a possible extension for the thi year, and it will be made in the fi three steps of the Lecturer Scale.

The main emphasis in the S Science Section relates to physical a clerical aspects of soil/plant relatic ships and it is expected that the p son appointed would strengthen the interests: research experience is des able.

Further particulars and forms application, returnable not later th March 12, 1979, from the St Appointments Officer, University Nottingham, University Park, N tingham NG7 2RD. Ref. No. 675. 9766(A)

UNIVERSITY OF ST. ANDREWS

DEPARTMENT OF ANATOMY AND EXPERIMENTAL PATHOLOGY

Applications are invited for the post of

TECHNICIAN (GRADE 4)

in the above Department. The successful applicant will take charge of the dissecting room and embalming suite, participate in the preparation of material for display in the departmental museum and assist with the preparation of teaching material.

Salary at appropriate point on scale £2,955 to £3,402 per annum (under review).

Applications, with the names of two referees should be sent to The Establishments Officer, The University, College Gate, St. Andrews, Fife, by March 2, 1979. 9792(A)

UNIVERSITY OF DAR ES SALAAM TANZANIA

Applications are invited for the following posts in the

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

PROFESSOR/ASSOCIATE PROFESSOR IN FOOD SCIENCE

Applicants should have a good B.Sc. in Food Science and Ph.D. in any area of Food Science and Technology with wide teaching and research experience at University level. Past experience in setting up and organising new Food Science Laboratories would be of advantage. The appointee would be required to teach in his/her area of speciality in an integrated undergraduate training programme for B.Sc. (Food Science) and B.Sc. (Agric) courses. The appointee would also be required to initiate and supervise research projects in the area of Food Science both at undergraduate and postgraduate levels.

LECTURER/SENIOR LECTURER IN FOOD ENGINEERING AND FOOD MANUFACTURE

Applicants should have a good B.Sc. in Food Science and Ph.D. in Food Engineering or Food Manufacture with considerable teaching and research experience at University level. Past experience in setting up new Food Science Laboratories would be advantageous. The appointee would be required to teach all aspects of Food Engineering and Food Manufacture to undergraduate students studying for B.Sc. (Food Science) and B.Sc. (Agric). The appointee would also be expected to initiate and supervise research programmes in the area of Food Science both at graduate and postgraduate levels.

SENIOR LECTURER/LECTURER IN FOOD MICROBIOLOGY

Applicants should have a good B.Sc. in Food Science and a Ph.D. in Food Microbiology with considerable teaching and research experience at University level. Experience in setting up new Food Microbiology Laboratories would be advantageous. The appointee would be required to teach Food Microbiology to students studying for B.Sc. (Food Science) and B.Sc. (Agric). The appointee would also be required to initiate and supervise research work in the area of Food Microbiology at undergradute and postgraduate levels.

LECTURER IN FOOD ECONOMICS AND MANAGEMENT

Applicants should have a good B.Sc. in Food Science or Home Economics and Ph.D. in Food Economics and Management with considerable teaching and research experience at University level. The appointee will be required to teach Food Economics and Management to students studying for B.Sc. (Food Science) and B.Sc. (Agric). The appointee would also be required to initiate and supervise research work in Food Economics and Management both at graduate and postgraduate levels.

Salary Scales (Salaries are exclusive of 50% expatriation allowance): Professor £T2,700 p.a.; Associate Professor £T2,380 to £T2,580 p.a.; Senior Lecturer £T1,980 to £T2,480 p.a.; Lecturer £T1,620 to £T1,980 p.a. (£T1=£1.22 sterling.) The British Government may supplement salaries in range £4,356 to £5,964 p.a. (sterling) for married appointees and £2,754 to £4,038 p.a. (sterling) for single appointees (reviewed annually and normally free of tax) and provide children's education allowances and holiday visit passages. Family pasages; F.S.S.U.; biennial overseas leave.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Chief Academic Officer, University of Dar es Salaam, P.O. Box 35091, Dar es Salaam, Tanzania, by April 8, 1979.

Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9796(A)

UNIVERSITY OF CALIFORNIA, BERKELEY

DEPARTMENT OF
ENTOMOLOGICAL SCIENCES,
COLLEGE OF
NATURAL RESOURCES

FACULTY POSITION IN INSECT PATHOLOGY

Full-time, 11-month research (75 per cent) and teaching (25 per cent) position available in insect pathology at the Assistant Professor level available July 1, 1979. Expertise mandatory in one of the following areas: bacteriology, comparative virology, mycology, or protozoology. Ph.D. and background in pathology required and postdoctoral experience desirable.

Responsibilities will be in basic and applied research and teaching in insect pathology and pest management. Experience in pathogen-insect relationships, pathogenicity, and the manipulation of pathogens for insect control will be weighting factors when judging applicants.

Send full curriculum vitae, official transcripts, copies of significant publications or manuscripts, and names of three references by April 1, 1979 to Evert I. Schlinger, Acting Chairman, Department of Entomological Sciences, 137 Giannini Hall, University of California, Berkeley, California 94720. The University of California is an Equal Opportunity/Affirmative Action Employer. 9755(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited
for the post of

SENIOR LECTURER

in the
DEPARTMENT OF PHARMACY

Applicants must have a good degree in Pharmacy and a Ph.D. in Pharmaceutics. In addition the applicants are expected to have teaching and research experience at University level. Those with postgraduate qualifications in related branches of Pharmacy will also be considered. Salary Scale: K£2,988 to K£3,984 per annum (K£1 equals £1 sterling). The British Government may supplement salary by £5,430 per annum (sterling) for married appointee or £3,648 per annum (sterling) for single appointee (reviewed annually and normally free of tax) and provide children's education allowances and holiday visit passages. Family passages; superannuation scheme; medical aid scheme; various allowances. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent direct to Registrar, University of Nairobi, P.O. Box 30197, Nairobi, Kenya by April 5, 1979. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9759(A)

Industrial Scientist

Senior Scientific Officer/Higher Scientific Officer

Applications are invited for the above pensionable post in the Industrial Science Division of the Department of Commerce situated at 17 Antrim Road, Lisburn, Co Antrim.

The successful candidate will work in a section which is involved in the continuous survey of international innovation and the technical appraisal of research and development projects.

The duties will involve the vetting of submitted projects and the evaluation of them in the context of existing technology or innovations to be found in patent and other literature.

The Industrial Science Division offers Industry and Government a wide range of scientific and technical services to aid in legislative administration, production, quality control and research and development.

The Division has substantial library facilities for the use of the successful candidate, and the services of the Information Officer may be called upon to survey the literature by means of computer links with UK and foreign data bases.

Appointment may be made at either Senior Scientific Officer or Higher Scientific Officer.

Applicants should normally have had some experience of Research and Development in either the pure or applied fields and preferably be qualified as chemists, chemical engineers, chemical technologists or physicists. Applicants must have industrial experience preferably in more than one manufacturing situation.

SENIOR SCIENTIFIC OFFICER

Over 25 and under 32 years of age on 31 December 1979 and possess a 1st or 2nd class honours degree in Chemistry, Physics, Chemical Engineering or Chemical Technology and have at least 4 years' appropriate post-graduate experience as outlined above.

HIGHER SCIENTIFIC OFFICER

Under 30 years of age on 31 December 1979 and possess an Honours degree in Chemistry, Physics, Chemical Engineering or Chemical Technology and have at least 2 years' appropriate post-graduate experience as outlined above.

Exceptionally, applications may be considered from candidates over the age limit who have specialised experience.

The Commissioners may decide to interview only those applicants who appear from the information available (including level of academic attainments and experience) to be best qualified.

SALARY SCALES

Senior Scientific Officer—£5,154-£6,898
Higher Scientific Officer—£4,101-£5,448

The salary will be subject to review with effect from 1 April 1979.

Grading and Starting salary will be related to qualifications and experience.

Please write or telephone for an application form quoting job reference SB 77/79/NA to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext. 256). Completed forms must be returned to arrive not later than 8 March, 1979.

9707(A)

NORTHERN IRELAND CIVIL SERVICE

MEDICAL LAB TECHNICIAN

Graduate preferred, trained in light and transmission electron microscopy, required as soon as possible to work in Oxford. Knowledge of day-to-day maintenance of an up-to-date electron microscope an advantage. Manufacturers contract for regular major service and breakdowns maintained. Initial three year contract on current Whitely Council scales.

Apply for further details to: Director, L.E.P.R.A., Fairfax House, Colchester. 9704(A)

UNIVERSITY OF DAR ES SALAAM
TANZANIA

Applications are invited for the following posts in the

DEPARTMENT OF AGRICULTURAL ENGINEERING AND LAND PLANNING

LECTURER/SENIOR LECTURER/ASSOCIATE PROFESSOR IN SOIL AND WATER ENGINEERING

Candidates should possess B.Sc. in Agricultural Engineering, Engineering, Agriculture with engineering specialisation or Geography together with M.Sc. and/or Ph.D. with emphasis on Soil and Water Engineering; and/or Land Planning. Practical experience with responsibility in Land Planning and management for arable and livestock farming; soil conservation and/or irrigation is desirable, as is teaching experience. The appointee will be required to teach in the B.Sc. and M.Sc. programmes in the general area of Soil and Water Engineering with emphasis on Land evaluation, Land capability, classification and Land development for agricultural production including arable and livestock farming. He/she will be expected to supervise B.Sc. and M.Sc. work and undertake research in this area of study, and develop liaison with field staff in government and parastatal organisations.

LECTURER/SENIOR LECTURER/ASSOCIATE PROFESSOR IN PROCESSING AND STORAGE OF AGRICULTURAL CROPS

Candidates should possess a B.Sc. in Agricultural Engineering, Agriculture or Engineering preferably together with M.Sc. in a relevant specialist study. Responsible practical and/or research experience is also required and teaching experience would be an advantage. The appointee will be required to teach in B.Sc. and M.Sc. programmes in the general area of farm village and estate level processing of foodstuffs including crop drying and storage, processing of dairy products and crops of economic importance such as coffee, cotton, sisal, sugar. He/she will be required to supervise students undertaking special studies; participate in the Department's research programme; collaborate with and provide advice to units within and outside the University.

LECTURER/SENIOR LECTURER/ASSOCIATE PROFESSOR IN FARM AND RURAL STRUCTURE

Candidates should possess a B.Sc. in Agricultural Engineering, Agriculture or Engineering preferably with M.Sc. or Ph.D. in a relevant area of study. Responsible practical and/or research experience is also required and teaching experience is desirable. The appointee will be required to teach in B.Sc. and M.Sc. programmes in the general area of functional and constructional aspects of structures for livestock, storage and processing; layout of buildings and equipment and provision of service. Particular attention is given in the Department to requirements at farm and village level. It is anticipated that the expert will disseminate sound practice in the field through liaison with rural communities and advisory workers. He/she will be expected to undertake research and development work.

LECTURER/SENIOR LECTURER/ASSOCIATE PROFESSOR IN FARM POWER AND MECHANISATION

Candidates should be holders of B.Sc. in Agricultural Engineering or in Agriculture with strong mechanisation option and responsible experience in the application and management of farm machinery in agricultural producton. A relevant M.Sc. or Ph.D. is highly desirable, as is teaching experience. The appointee will be required to teach in the B.Sc. and M.Sc. programmes in the general area of farm power and machinery management, and to supervise students undertaking special duties. He/she will be expected to contribute to the Department's research programme and work in conjunction with other units within and outside the University when appropriate.

Salary scales (salaries exclusive of 50% expatriation allowance): Associate Professor £T2,380 to £T2,580 p.a.; Senior Lecturer £T1,980 to £T2,480 p.a.; Lecturer £T1,620 to £T1,980 p.a. (£T1=£1.22 sterling.) The British Government may supplement salaries in range £4,356 to £5,220 p.a. (sterling) for married appointees and £2,754 to £3,330 p.a. (sterling) for single appointees (reviewed annually and normally free of tax) and provide children's education allowances and holiday visit passages. Family passages; F.S.S.U.; biennial overseas leave.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Chief Academic Officer, University of Dar es Salaam, P.O. Box 35091, Dar es Salaam, Tanzania, by April 8, 1979.

Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9797(A)

CHARING CROSS HOSPITAL
SENIOR MEDICAL LABORATORY SCIENTIFIC OFFICER

required for Tissue Typing Section of our Haematology Department. State Registration and experience in HLA Typing essential. For further details, please ring Mr T. Fletcher, Senior Chief Medical Laboratory Scientific Officer, Haematology, on (01)-748 2040, Bleep 91.

Application forms from District Personnel Department,
Charing Cross Hospital, Fulham Palace Road,
Hammersmith, London W6
Tel: (01)-748 2040, ext. 2992/3021

9790(A)

UNIVERSITY OF DUBLIN
Trinity College

DEPARTMENT OF PHYSIOLOGY
LECTURER

Applications are invited from medical, dental or science graduates for this position which carries main responsibility for teaching in the following courses:

Histology (some general and all special) to Dental students.

Histology to Pharmacy Students.

Anatomy and Physiology: a co-ordinated course to students of Remedial Linguistics (Speech Therapy).

A research interest is essential in an area or areas related to the teaching programmes.

Salary scale: £4,232 to £8,321 per annum. Appointment is likely to be made within the range £4,232 to £4,680.

Application forms and further particulars relating to this post may be obtained from:

The Establishment Officer,
The Establishment Officer,
West Theatre,
Trinity College,
Dublin 2

to whom completed applications should be sent **not later than March 14, 1979.**

9706(A)

CHARING CROSS HOSPITAL MEDICAL SCHOOL
(University of London)
DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral Research Assistant interested in reproductive biology with specific reference to the placenta. The applicants will be expected to have experience in membrane biochemistry, immunology and cell biology. Salary on scale £3,883 to £6,555 plus £450 London Allowance. Further details obtainable from Dr Contractor, 01-748 3441, extension 127.

Applications including full curriculum vitae and names of two referees should be sent to The Secretary, Charing Cross Hospital Medical School, The Reynold's Building, St Dunstan's Road, London W6 8RP. (Ref: 470/1).

9771(A)

MEDICAL RESEARCH COUNCIL
DEVELOPMENTAL NEUROBIOLOGY UNIT
INSTITUTE OF NEUROLOGY

Applications are invited from young postdoctoral morphologists to participate in a programme to study effects of metabolic factors on brain development. Research experience in quantitative morphological methods, light and electron-microscopic autoradiographic, and immunohistochemical techniques will be of advantage. The appointment will be for one year in the first instance with the possibility of extension for a further two years. Salary will be at the appropriate point on the MRC scale for non-clinical scientific staff.

Applicants should send a Curriculum Vitae giving full details of qualifications and experience, and the names of two referees to: Dr R. Balazs, MRC Developmental Neurobiology Unit, Institute of Neurology, 33 John's Mews, London WC1N 2NS. 9734(A)

UNIVERSITY OF CALIFORNIA, BERKELEY
DEPARTMENT OF ENTOMOLOGICAL SCIENCES, COLLEGE OF NATURAL RESOURCES
FACULTY POSITION IN NATURAL PRODUCTS CHEMISTRY

Full-time, 11-month research (80 per cent) and teaching (20 per cent) position available in Natural Products Chemistry at the Assistant Professor level. Ph.D. in organic chemistry or other fields(s) relevant to natural products required with evidence of interest in chemicals active in insects.

Responsibilities will be in basic and applied research involving bioactive chemicals from insects, plants, and other biological materials that influence insect growth, development, reproduction, and behaviour. Research will include identification and synthesis of active components, and studies on their potential use in managing insect populations. Teaching duties may include developing a course in chemical ecology, environmental chemistry, insect biochemistry, or chemical pest control.

Send full curriculum vitae, official transcripts, copies of significant publications or manuscripts, and names of at least three references by April 1, 1979 to Evert I. Schlinger, Acting Chairman, Department of Entomological Sciences, 137 Giannini Hall, University of California, Berkeley, California 94720. The University of California is an Equal Opportunity/Affirmative Action Employer.

9756(A)

Biochemistry Technician required for research project involving glycoprotein separation and metabolism in calcified tissues. Experience with chromatographic, immunochemical and radioisotopic techniques useful, but not essential. Salary on Whitley Council Medical Laboratory Scientific Officer scale £3,261 to £4,680 per annum, according to age, qualifications and experience. Apply giving curriculum vitae and the names of two referees to: Dr J. T. Triffitt, M.R.C. Bone Research Laboratory, Nuffield Department of Orthopaedic Surgery, University of Oxford, Nuffield Orthopaedic Centre, Oxford OX3 7LD. 9772(A)

TWO NON-MEDICAL RESEARCH ASSISTANTS

required in the Host Defence Unit of the Department of Medicine, Cardiothoracic Institute, to assist in a three-year project investigating phagocyte function and muco-ciliary clearance mechanisms in human and animal respiratory infections. Practical experience in either humoral/cellular immunology, electron microscopy, or cell physiology/biochemistry essential.

Applications, stating age, qualifications and giving details of practical experience, with particular reference to the areas stated, together with the names and addresses of two referees to R. A. Perkins, Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP. Please quote reference HDU 1/79. 9773(A)

LABORATORY SCIENTIFIC OFFICER required in Department of Oral Immunology and Microbiology for three years, for research into immunity to Streptococci in man and animals. Experience in immunology and/or microbiology desirable. Salary for qualified Laboratory Scientific Officer not less than £3,261, on scale rising to £3,930, plus £354 London Weighting. Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. I.M.I.

9794(A)

THE WEST OF SCOTLAND AGRICULTURAL COLLEGE
AGRICULTURAL CHEMISTRY DIVISION

SPECIALIST ADVISER ANALYTICAL CHEMISTRY)

Applications are invited for a post of Specialist Adviser (Analytical Chemistry) in the Division's Analytical Services Unit. Applicants should have a degree or equivalent qualification in Chemistry or Agricultural Chemistry. Experience in the establishment operation and control of large scale analyical procedures woud be an advantage.

The salary will, according to age, qualifications and experience, be on one or other of the following scales:-

Grade IV—£2,998 (Honours — 1st or 2nd Class — £3,167) rising to £3,924

III—£4,178 riisng to £6,898

Further particulars and application forms may be obtained from the Secreary, the West of Scotland Agricultural College, Auchincruive, Ayr KA6 5HW with whom applications should be odged not later than March 2, 1979.

9744(A)

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for the post of **SRC** Postdoctoral Research Associate to work on the kinetics of the coupled transport of β-galactosides and protons into *Escherichia coli*. Applicants with experience of pre-steady-state kinetics are particularly wanted but experience in the fields of membrane transport or bioenergetics would also be considered relevant. Further details may be obtained from Dr I. C. West.

The apopintment will be for three years, starting as soon as possible. The starting will be at the appropriate point on the 1A Research Associate scale (£4,382 at age 26, under review).

Applications, including a *curriculum vitae* and the names of two referees, should be sent to the Administrative Assistant, Department of Biochemistry, The University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU.

9745(A)

THE ROYAL FREE HOSPITAL HAMPSTEAD

MEDICAL PHYSICS TECHNICIAN GRADE I

Salary Scale: £5,787 to £6,576 per annum (including all allowances)

To be in charge of large Inter-departmental Mechanical Workshops based in one of London's largest and most modern Hospital/Medical School complexes.

The Workshop is responsible for making scientific and research equipment required by a variety of Hospital and Medical School Departments.

The successful applicant will have had extensive closely related experience in a multi-user Department and have proven ability to supervise the work of junior staff.

Candidates must possess either a recognised formal qualification or a minimum of 9 years' experience in a relevant field.

Application form (to be returned by March 7, 1979) and Job Description available from the Personnel Departments, The Royal Free Hospital, Pond Street, Hampstead, London, NW3 2OG. Telephone: 794-0500 Ext. 4286. Please quote ref: 0762.

Camden and Islington Area Health Authority (T).

9718(A)

OVERSEAS DEVELOPMENT

KNOW-HOW: vital to developing countries

Agricultural Entomologist - Tropical Agricultural Mission Bolivia

To work with Sugar Cane Growers Association's (CIMCA) research team on research station north of Santa Cruz. To carry out an integrated control programme on pests in sugar cane involving both insecticidal and biological control methods in addition to testing of resistant material. Will also be required to train local staff in sugar cane pest control methods.

Appointment 2 years, salary (UK taxable) in range £6,400 to £8,400 p.a., plus variable tax-free overseas allowance in range £1,440 to £2,825 p.a. (Ref. 331X)

Crop Physiologist Botswana

To work as part of a 3-man team including Tropical Agronomist and Soil Physicist for a Dryland Farming Research Scheme—Phase III, situated at the Agricultural Research Station, Gaborone, Botswana.

The crop physiologist will study the edaphic and aerial factors which are optimum for crop growth (mainly sorghum). This work will include the study of water and energy balances of the sorghum crop by analysing the climate in relation to crop growth, water use and also the soil physical properties. Specific studies are also necessary on the role of night temperatures on grain development and yield. The successful candidate will work to a Team Leader chosen from the Team. Applicants should have a degree in science or agricultural science with research experience in plant physiology and preferably overseas experience.

Appointment 3 years, salary (UK taxable) in range £5,700 to £9,700 p.a., plus tax free overseas allowance in range £1,360 to £3,375 p.a. (Ref. 331X).

These posts are wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, childrens education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, quoting reference stating post concerned, and giving details of age, qualifications and experience to:—

ODM

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London SW1E 5DH.

9764(A)

HELPING NATIONS HELP THEMSELVES

Plant Protection Division Jealott's Hill Research Station have the following vacancies in the Botany Section of the Biological Research Group:

Technical Officer

Plant Physiologist–Ref DS/Bot 1

A plant physiologist with PhD or equivalent qualification is required to assist in the discovery and evaluation of new herbicides. Experience of experimental work in controlled environment rooms and under glasshouse conditions is desirable. The person appointed will join a multidisciplinary project team and will be responsible for the work of two small experimental teams which include graduate level staff.

Experimental Officer

Plant Physiologist–Ref SRS/Bot 2

A graduate plant physiologist with 2/3 years' relevant experience is required to join a team carrying out experiments in controlled environment rooms and glasshouses on new herbicides The job holder will supervise a team of one or two assistants and will respond to the Technical Officer with overall responsibility for the work programme.

Experimental Officer

Field Trials–Ref SRS/Bot 3

A graduate with a degree in Botany or Agricultural Sciences and at least two years' practical experience of field experimentation is required to organise and perform the initial field screening of Plant Growth Regulators. The person appointed will respond to a Technical Officer with overall responsibility for the work programme.

Jealott's Hill Research Station is situated on a pleasant country estate between Bracknell and Maidenhead and subsidised coaches run to and from Jealott's Hill and these two towns We offer good salaries and fringe benefits include membership of the ICI Profit Sharing Scheme and Pension Fund. We have a good self service restaurant and recreation club on site.

If you are interested in any of these vacancies please write for an application form quoting reference number to:

Personnel Officer
ICI Plant Protection Division
Jealott's Hill Station
Bracknell
Berkshire

9719(A)

ICI Plant Protection Division

UNIVERSITY OF DAR ES SALAAM TANZANIA

Applications are invited for the following posts in the

DEPARTMENT OF GEOGRAPHY

LECTURER/SENIOR LECTURER IN REGIONAL PLANNING

Applicants should preferably have a Ph.D. in the relevant area In addition they should have research and teaching experience in rural/regional planning in the Third World. The appointee will be required to teach two undergraduate courses and at least one postgraduate course in Urban and regional planning as well as supervise research related to his/her field of specialisation.

LECTURER/SENIOR LECTURER IN BIOGEOGRAPHY

Applicants should have a postgraduate training to Ph.D. level preferably with research experience in tropical soils and vegetation The appointee will be expected to teach two undergraduate courses in soils and vegetation, tropical ecosystems and environmental management. In addition he/she will be expected to teach a postgraduate course. 'Assessment and use of the environment in underdeveloped Economies' and carry out research in his/her field of specialisation

LECTURER/SENIOR LECTURER IN URBAN GEOGRAPHY

Applicants should have a Ph D., as well as experience in teaching and research A radical geographical orientation will be an added advantage Duties will include teaching undergraduate courses in urban geography to second and third year students and organisation of seminars and research projects for postgraduate students

Salary scales: (Salaries exclusive of 50 per cent expatriation allowance), Senior Lecturer. £T1,980 to £T2,480 per annum, Lecturer £T1,620 to £T1,980 per annum (£T1 equals £1 22 sterling). The British Government may supplement salaries in range £4,356 to £4,782 per annum (sterling) for married appointees and £2,754 to £3,018 per annum (sterling) for single appointees (reviewed annually and normally free of tax) and provide children's education allowances and holiday visit passages. Family passages, F S S U, biennial overseas leave Detailed applications (two copies) with curriculum vitae and naming three referees to be sent direct to Chief Academic Officer, University of Dar es Salaam, P.O. Box 30197, Dar es Salaam, Tanzania by April 8, 1979 Applicants resident in the U K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address

9758(A)

MONASH UNIVERSITY

Melbourne, Australia

CHAIR OF PHYSIOLOG

Monash University is seeking t the position of Professor of Phys which became vacant on the retire of Professor A K McIntyre The Professor will be expected to as Chairmanship of the Departmen arrangement with the present C man and take the leadership rol organising the teaching and res functions of the Department

The Department has recently m into a new building providing 4,0 of net space well equipped for teac and research activities.

The Department teaches extens in the Faculties of Medicine Science There is a significant Ho programme and graduate school ponent of the Department's teac activities.

The present research interests o Department are in neuropharmaco neurophysiology, endocrinology, structure and muscle physiology. appointee should be an establi scientist whose field of interest c be within or complementary to on these areas

Detailed information about the partment can be obtained from Chairman, Professor R Porter, in University and persons interested invited to write to him

Salary $A33,061 per annum pre-clinical loading of $A2,500 annum will be paid to a medi qualified appointee Superannua Travel and removal allowance

Application procedure and fu particulars available from the Acad Registrar, Monash University, Cla Victoria, 3168, Australia, or the S tary General, Association of Com wealth Universities (Appts) 36 Go Square, London WC1H 0PF

Applications should reach Academic Registrar not later June 30, 1979. The Council rese the right to make no appointmen to appoint by invitation at any s

9804(

AUSTRALIAN NATIONAL UNIVERSIT

THE JOHN CURTIN SCHOOL MEDICAL RESEARCH

DIRECTOR

The University seeks to appoi Head of the John Curtin School Medical Research to succeed Prof F. Gibson, FAA, FRS, whose cur term as Director expires on Dece 31, 1979, and who does not seek extension of his appointment Director

The Head of a Research Scho responsible through the Vice-Chanc to the University Council for operation and development of School and for its administration financial management

The Director should have acad experience within the discip appropriate to the School and preferably be medically qualified present interests of the School emb biochemistry, clinical science, ex mental pathology, human bio immunology, medical chemistry, m biology, pharmacology, physical chemistry and physiology Staff engaged in research and the tra of postgraduate students the Sc has no undergraduate students

The Director will also hold a C in the University The term of o as Director will be a matter for a ment with the University but is normally for less than three years more than seven, with the possib of extension for a further pe Appointment as professor is retiring age, i e the end of the in which the professor reaches the of 65 years, with the option of ret at any age after age 60

Those who may be interested being considered for this appoint are invited to write, as soon possible to the Vice-Chancellor, Australian National University, Box 4, Canberra, ACT 2600, Austr from whom further information be obtained

G E Dicker, Registr

9800(

AGRICULTURAL RESEARCH COUNCIL

Institute of Animal Physiology

Babraham, Cambridge CB2 4AT

A vacancy exists in the Department of Cell Biology for someone to carry out basic research on developmental problems of intestinal physiology or on ultrastructural aspects of cell function. The person appointed will be expected to have already made a significant contribution to research using one or more of the following techniques: cell separation and culture, immunocytochemistry, membrane biochemistry or electron microscopy Applicants should have a first class or upper second class honours degree in a relevant subject with at least four years post-qualifying experience Preference for Ph D Appointment in grade of Senior Scientific Officer (£5,154 to £6,898 per annum) 'Non-contributory superannuation scheme. Application form and further details may be obtained from the Secretary of the Institute Quote reference C W.R 6 Closing date. March 12, 1979

9738(A)

DEPARTMENT OF GEOLOGY

DALHOUSIE UNIVERSITY

Halifax, Nova Scotia

The Department has a vacancy for a geologist with broad interests, preferably in the general fields of mineralogy, petrology, structural geology, economic geology, or geochemistry He or she would be expected to play a full part in teaching at the undergraduate and graduate level, and in research programmes with graduate students The Department covers a wide range of interests and has good facilities It carries out cooperative work with the N S Department of Mines and has close ties with the Bedford Institute of Oceanography The appointment will be made as soon as possible at the Assistant or Associate Professor level, with appropriate salary Canadians studying abroad are urged to apply Further details may be obtained from the Acting Chairman, Department of Geology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 3J5

9747(A)

THE CITY UNIVERSITY

CHEMICAL ENERGY RESEARCH UNIT

POSTDOCTORAL SCIENTISTS

for staff positions in the above Unit The objective of the Unit is to provide research and consultancy services on energy to industry and government departments Candidates should have research experience in one of the following areas Electrochemistry, Corrosion Science, Gas Phase Catalysis, Solid State Chemistry

Salary within the range £3,883 to £5,842 per annum plus £450 London Allowance and U S S benefits Applications, together with the names of two referees should be sent to Dr A C C Tseung, Director, Chemical Energy Research Unit, The City University, Northampton Square, London EC1V 0HB

9762(A)

Ministry of Agriculture Fisheries and Food

ROYAL BOTANIC GARDENS
WAKEHURST PLACE, ARDINGLY, SUSSEX

Forest Tree Seed Physiologist

required to investigate the physiology of loss of viability in stored seeds of tropical forest trees with a view to overcoming the present difficulties encountered in their collection, transportation and storage. The work will involve determining and quantifying the "orthodox" or "recalcitrant" nature of their storage physiology; this aspect will be carried out at Wakehurst Place. The development of the results into practical and proven techniques will necessitate considerable periods of individual field work in the latter part of the project. Close co-operation with the Commonwealth Forestry Institute in the selection of species for investigation is essential. The appointment will be for a period of 3 years.

QUALIFICATIONS: An honours degree in botany, biology or forestry with at least 2 years relevant post graduate experience.

AGE: Applicants should preferably be under 30 years of age on 31 December 1979.

SALARY: Will be at higher scientific officer level—£4,101 rising to £4,415 per annum.

Application forms (for return by 8 March 1979) can be obtained from Miss S. Stephens, Ministry of Agriculture, Fisheries and Food, Room 504, Victory House, 30-34 Kingsway, London WC2B 6TU (telephone: 01-405 4310 extn. 318 or 319). 9736(A)

UNIVERSITY OF SURREY
DEPARTMENT OF BIOCHEMISTRY
RESEARCH FELLOW

Applications are invited for a posiion to work with Dr R. S. Jones and rofessor D. V. W. Parke on an A.R.C. upported project concerned with an spect of environmental carcinogenesis. Applicants (male or female) should be raduates of either chemistry or biohemistry and possess a Ph.D./D.Phil.

The appointment is for a period of years and will commence as soon as ossible at a starting salary within the ange of £3,883 to £6,553 per annum Range 1A).

Applications, in the form of a curriulum vitae, including the names and ddresses of two referees, should be ent to the Assistant Secretary (Peronnel), University of Surrey, Guildord, Surrey, GU2 5XH, to be received y March 15, 1979.

Further information may be obtained rom Dr R. S. Jones, in the Departnent of Biochemistry. 9728(A)

UNIVERSITY OF SALFORD
DEPARTMENT OF CHEMISTRY AND APPLIED CHEMISTRY
POSTDOCTORAL RESEARCH FELLOW

Required for a project sponsored by Pfizer Central Research, entitled 'Studies on the Synthesis of novel beta—Lactam Antibiotics' under the supervision of Dr S. M. Roberts. The appointment is tenable for one year in the first instance, commencing October 1, 1979.

Commencing salary within the range:— £3,883 to £4,382 p.a. (under review) USS.

Application forms obtainable from the Registrar, University of Salford, Salford M5 4WT (tel: 061-736 5843 ext. 215) to whom completed applications should be returned by not later than March 31, 1979, quoting reference no. CH/254/N. 9776(A)

UNIVERSITY OF ESSEX
Department of Chemistry
TEMPORARY LECTURESHIP IN ORGANIC CHEMISTRY

Applications are invited for a one-year Lectureship commencing October 1, 1979 on salary scale £3,883 to £7,754 (under review).

Applications (two copies), including a curriculum vitae and the names and addresses of two academic referees, should reach the Registrar (AG/69/N), University of Essex, Wivenhoe Park, Colchester CO4 3SQ, from whom further particulars may be obtained, by March 31, 1979. 9717(A)

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
RESEARCH TECHNICIAN

Applications are invited for the post of Research Technician within a small team investigating the physical biochemistry of connective tissue, under a programme grant which is supported by the M.R.C. Candidates should be in possession of City and Guilds, H.N.C. or equivalent in chemistry and should have had experience in quantitative analysis. Inclusive initial salary related to qualifications and experience will be £3,711 to £4,263 per annum.

Enquiries to Dr A. Maroudas, Tel: 01-247 5454, Ext. 421. Application forms from The Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, quoting reference RTBJU/2/79. 9727(A)

POSTDOCTORAL POSITION for work on the reticulo-stimulating effect of vaccines from anaerobic coryneforms C. *parvum* vaccines). Applicants should have major interest in biochemistry and cell fractionation procedures. Send curriculum vitae and names and addresses of three referees to Dr C. S. Cummins, Anaerobe Laboratory, Viginia Polytechnic Institute and State University, Blacksburg, Va. 24061. An Equal Opportunity/Affirmative Action Employer.

9737(A)

Ministry of Agriculture, Fisheries and Food
Food Science Division, London

Chemist/ Biochemist

This post is in the Food Surveillance Section, which assists the Division in providing an advisory service on all aspects of food science, food technology and human nutrition, both at home and abroad.

The chemist/biochemist appointed will act as scientific secretary to the Steering Group on Food Surveillance and working parties on the Monitoring of Foodstuffs for Heavy Metals. The work will involve administering food surveillance programmes designed to determine the incidence of contaminants in food, identifying areas of future observation, preparing reviews and committee papers and presenting data in official reports.

Candidates, normally aged under 32, should have a good honours degree in chemistry, biochemistry or closely related discipline. At least 4 years' post-graduate experience, preferably including knowledge of mycology, analytical chemistry, food technology and toxicology, is essential. The ability to prepare technical papers and reports advantageous.

Appointment as Senior Scientific Officer (£5620–£7360) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 23 March 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref: SB/36/AE.*

Department of Agriculture and Fisheries for Scotland, East Craigs, Edinburgh

Agricultural Botanist

. . . to join a small team testing new varieties of fodder crop species to establish their distinctness, uniformity and stability. The agriculturalist appointed will assess the field performance of new species under Scottish conditions and undertake statutory certification of seed stocks.

Candidates, normally aged under 30, should have a good honours degree or equivalent in an appropriate discipline. At least 2 years' relevant post-graduate experience is essential. An interest in modern taxonomic methods and experience of herbage variety testing is highly desirable.

Appointment as Higher Scientific Officer (£4100–£5450) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 23 March 1979) write to Scottish Office, Personnel Division, PM(PTS)3, Room 404, 16 Waterloo Place, Edinburgh, or telephone Edinburgh 031-557 8400 ext 4311/5088. *Please quote ref: SA/18/DLA.*

ALL THESE POSTS
ARE OPEN TO MEN AND WOMEN.

Science Group
CIVIL SERVICE

9802(A)

Open to both
men and women

Public Service Canada
Fonction publique Canada

PHYSICAL OCEANOGRAPHER
(109-025-026)

Salary: $16,500 – $34,045
Ref. No: 78-NCRSO-32-143 (N)
Fisheries and the Environment
Ocean and Aquatic Sciences
Bedford Institute of Oceanography
Dartmouth, Nova Scotia

The successful candidate will plan and conduct research on the physical processes which determine biological productivity on continental shelves, with a special emphasis on the role of tidal dynamics. The duties will involve participation in work at sea and extensive use of numerical simulation and analysis techniques.

Qualifications

Applicants must possess a Ph.D., or a lesser degree with evidence of research experience and productivity to a doctorate, in physical oceanography, physics, applied mathematics or a related field. Research experience and a demonstrated capacity to make original contributions in the study of continental shelf oceanography, particularly in the area of tidally-included shallow-water frontal systems, is required. Knowledge of English is essential.

How to Apply

Send your application form and/or résumé to:
D.E. Fraser
National Capital Region Staffing Office
Public Service Commission of Canada
Ottawa, Ontario K1A 0M7
Closing Date: March 26, 1979

Please quote the applicable reference number at all times.

9705(A)

UNIVERSITY OF OXFORD
UNIVERSITY LECTURESHIP IN CLINICAL MEDICINE

Applications are invited from medically-qualified candidates for the above post (vacant from September 1, 1979). Stipend on the scale £6,591 to £7,696; an honorary consultant contract may be available for a suitably qualified appointee in which case the stipend will be on the scale £9,528 to £12,084. The successful candidate will be expected to work in the field of immunology. Applications (seven typed copies, one from overseas applicants) to Professor D. J. Weatherall, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford, from whom further particulars may be obtained, by April 1, 1979. 9715(A)

QUEEN MARY COLLEGE
University of London
APPLIED MATHEMATICS DEPARTMENT

Applications are invited for appointment as

LECTURER

from October 1. Preference may be given to applicants whose research interests complement those of existing members of staff or the interests of members of other Departments in the College. Salary scale £3,975 to £8,250 p.a. (under review) plus £450 London Allowance. Application forms and further details available from The Registrar, (N) Queen Mary College, Mile End Road, London E1 4NS, to be returned by March 26.

9723(A)

FLINDERS MEDICAL CENTRE
Adelaide, South Australia
HOSPITAL SCIENTIST OIC ROUTINE MICROBIOLOGY

Flinders Medical Centre is an integrated university medical school and teaching hospital of 500 beds. The DEPARTMENT OF CLINICAL MICROBIOLOGY with a staff of 25 and headed by Assoc. Prof. P. J. McDonald is responsible for diagnostic services as well as clinical microbiology teaching and research with the School of Medicine, Flinders University.

The Department is presently divided into several sections including serology, virology, chlamydia, anerobic and cellular research and routine microbiology. Applications are invited for the position of OFFICER-IN-CHARGE — ROUTINE MICROBIOLOGY. This section, the largest in the Department, handles the routine diagnostic areas of bacteriology (including anaerobes and T.B.), parasitology and mycology. Approximately 3000 specimens are processed monthly.

The successful appicant will have had several years' experience in medical microbiology including diagnostic, teaching and developmental research responsibilities and by keeping abreast of current literature will be innovative in approach to diagnostic methods. The position itself carries responsibilities in the area of routine methods, evaluative projects and in-service training of staff as well as participation in the teaching of medical students and the departments research programs.

Salary is within the range $A10,891 to $A17,331 and it is expected the appointment will be made at the upper end of the range. After an initial period, personal reclassification to a higher scale is possible. A proportion of removal expenses will be provided.

Applications stating full curriculum vitae and confidential letters of recommendation pertaining to the position from two referees should reach The Administrator, Flinders Medical Centre, Bedford Park, South Australia, **5042** no later than March 27.

Further enquiries should be directed to the Senior Microbiologist, Mr T. R. Grimmond. Telephone 03-2759911. 9750(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
POSTDOCTORAL RESEARCH ASSISTANT IN BIOCHEMISTRY

Applications are invited for a postdoctoral biochemist to work in collaboration with Dr Anne Grant and Professor J. Hermon-Taylor on the biochemistry and surface components of human pancreatic and other cancer cells in tissue culture. The post, supported by the Cancer Research Campaign, is available immediately and runs until December 31, 1981 with the possibility of extension. Salary in the range £5,551 to £6,612 (under review). Further particulars and applications forms available from Professor J. Hermon-Taylor (01-672-1255 Ext. 4716) Department of Surgery, or the Establishment Officer, St George's Hospital Medical Schoil, Cranmer Terrace, London SW17 0RE. 9724(A)

POSTDOCTORAL POSITION IN MOLECULAR VIROLOGY. Available from April 1, 1979 to study early interaction between animal viruses and cells. Reply with resumé to: Dr Alfred T. H. Burness, Faculty of Medicine, Memorial University of Newfoundland, St John's Newfoundland, Canada, A1B 3V6. 9752(A)

LABORATORY SCIENTIFIC O CER, with H.N.C. or equiva qualification (or Junior Labora Scientific Officer of high qua required as soon as possible for year to complete an M.R.C.-suppo study on cytomegalovirus infection Department of Microbiology. Ex ence in virology or cell cul essential. Salary for qualified Lab tory Scientific Officer not less £3,261 plus £354 London Weight Applications with full details names of two referees to the Secret Guy's Hospital Medical School, Lon Bridge SE1 9RT, quoting Ref.
9795(

TECHNICIAN (Grade 6) required Department of Microbiology, versity of Reading, to supervise servicing of practical classes and production of microbiological m and sterile materials for teaching research. Salary in scale £3,654 £4,365 p.a. (under review). Apply further details quoting Ref. T.N. to Assistant Bursar (Personnel), versity of Reading, Whiteknig Reading, Berks, RG6 2AH. 9791(A

MEDICAL RESEARCH COUNCIL LABORATORY OF MOLECULAR BIOLO

Applications are invited for a requiring research on the structure chromatin. The person appointed be expected to pursue biochem studies as a member of a resea group working on the biochemistry three dimensional structure of nuc somes and higher order structu Experience in protein and nucleic a chemistry would be an advantage.

The appointment will be for th years in the first instance, start October 1, 1979. Candidates must h at least an upper second class hono degree and three years relevant p graduate experience.

Salary on scale starting from £4, per annum.

Applications, with full curricul vitae and names of two referees, sho be sent to the Administrative Sec tary, M.R.C. Laboratory of Molecu Biology, The Medical School, H Road, Cambridge CB2 2QH, to received not later than March 1979. 9806(A

THE UNIVERSITY OF MA CHESTER. There is a vacancy fo TECHNICIAN (Grade 5, Univer Scale) in the biochemical resea laboratories of the clinical dep ments at the Medical School (Stopf Building). Applicants should have least eight years experience in clinical chemistry or biochemical oratory and possess A.I.M.L.S. H.N.C. in appropriate subjects.

Salary scale (under review) £3, to £3,720 per annum.

Applications, quoting two refer should be sent as soon as possible Professor P. H. Adams, Department Medicine, Royal Infirmary, M chester M13 9WL from whom furt particulars may be obtained.
9760(A

UNIVERSITY OF GLASGO
SECOND CHAIR OF ZOOLOGY

The University invites applicati for a second chair in its Departm of Zoology.

Further particulars may be obtai from the Secretary of the Univers Court (Room 18), University Glasgow, Glasgow G12 8QQ, w whom applications (15 copies; copy in the case of overseas ap cants), giving the names and addres of three referees, should be lodged or before March 16, 1979.

In reply please quote Ref. 4270. 9710(A

MEDICAL RESEARCH COUNCIL
Radiobiology Unit, Harwell
MAMMALIAN GENETICIST OR CYTOGENETICIST

A scientist is needed to join a group studying the genetic effects of radiation and other mutagenic agents in mammals. The emphasis is on methods of exploring the sensitivity to mutagenesis of germ cells of different sexes, developmental stages and species in the intact animal Formal genetics and cytogenetics relevant to the mutagenesis work are also included Candidates with experience of genetics of higher organisms, or of handling rodent chromosomes, at either meiotic or mitotic stages, would be at an advantage.

Possible subjects of research include

(i) comparison of the sensitivity to mutagens of various mammalian species, with particular emphasis on methods of studying chromosome changes in oocytes and early embryos,

(ii) studies of the causes of chromosomal non-disjunction in mammals,

(iii) factors affecting the sensitivity of oocytes to mutagenesis.

There are excellent research facilities. The Unit also includes groups studying cell mutation, cytogenetics, pathology, and effects of radionuclides.

Applicants should hold a relevant good honours degree, with three year's research experience, and preferably a Ph.D. Salary and conditions of service according to M.R.C non-clinical scientific scales. Hostel accommodation for single, or housing for married, people may be available.

Applications, with curriculum vitae and names of two referees, should be sent to Administrator, M.R.C Radiobiology Unit, Harwell, Didcot, Oxon. OX11 0RD, from whom further particulars may be obtained. 9733(A)

UNIVERSITY OF BATH
BIOCHEMISTRY GROUP
RESEARCH OFFICER

required to work with Dr D. Hough on a S.R.C.-supported project to investigate 'The attachment of immunoglobulin to the membrane of human lymphocytes'. The work will include maintenance of lymphoid cell cultures, radiolabelling and radioimmunoassay of membrane immunoglobulins. Persons expecting to graduate in July may be considered for this post, which is available for two years

Commencing salary £3,384 per annum (under review).

Application forms from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting reference number 79/20/N. Closing date will be March 6, 1979 9708(A)

UNIVERSITY OF STIRLING
DEPARTMENT OF BIOCHEMISTRY
CHIEF TECHNICIAN

Applications are invited for the post of Chief Technician in Biochemistry The successful candidate will supervise the technical staff in the department and will be in charge of the teaching laboratories. Candidates should hold an HNC or equivalent qualification and should have experience in biochemistry or related area The post is tenable from April 1979 The salary will be within the Grade 6 scale £3,654 to £4,365 (under review)

Further particulars may be obtained from The University Secretary, University of Stirling, Stirling FK9 4LA

Closing date for applications March 7 9713(A)

Wellcome

Pathologist–Toxicologist

to join the Pathology Department of The Wellcome Research Laboratories located in Beckenham, Kent.

The Department is responsible for safety evaluation of new drugs and biologicals, including vaccines, for assessment and development of toxicological test methods, and it collaborates with other research workers at Beckenham and elsewhere within the Group The staff of the Department numbers about 70 people, including 12 graduates, and it has extensive facilities, including ample resources for many different types of research.

The successful applicant will report to the Head of the Department ,and take part in the routine work and research of the Department. Much of the work is based on morbid anatomy and experience of human or veterinary pathology is necessary. Knowledge of laboratory animal pathology would be advantageous, but it is not essential as further training could be given. Proven interest in research would be of value.

Applicants should have a medical or veterinary qualification and preferably a higher degree or qualification of the Royal College of Pathologists.

The Laboratories are situated in pleasant parkland surroundings within easy reach of Central London and conditions of employment are excellent and include subsidised canteen, pension scheme, 4 weeks' holiday, relocation expenses where appropriate and sports and social facilities.

Please write to

P. C. Anderson, Personnel Assistant,
The Wellcome Research Laboratories,
Langley Court,
Beckenham, Kent BR3 3BS. 9732(A)

1976

UNIVERSITY OF LONDON
KING'S COLLEGE
TECHNICIAN GRADE 3

Required for Electron Microscope Unit of the Anatomy Department to work under the direction of Grade 5 Technician The unit has an AEI EM 6B and a new JEOL CX100 with scanning facility and carries out work for other departments of the college Applicants should possess an O.N.C or equivalent qualification and have had three years' laboratory experience Salary on scale £3,153 per annum rising to £3,525 per annum inclusive (under review). Four weeks annual holiday Contributory pension scheme Apply in writing with full details to The Head Clerk (Ref 210230/N), King's College London, Strand WC2R 2LS. 9725(A)

UNIVERSITY OF LONDON
KING'S COLLEGE
RESEARCH TECHNICIAN GRADE 5

Required in Department of Physics, to work with Solid State Physics Group. Main responsibilities will be the maintenance, development and setting up of research equipment and preparation of samples Preference will be given to applicants with experience in either electronics or high vacuum and cryogenic techniques

Salary on scale £3,651 per annum inclusive (under review) rising to £4,185 per annum Four weeks annual holiday. Contributory pension scheme. Apply in writing with full details to: The Head Clerk (Ref 210232/N), King's College London, Strand WC2R 2LS 9726(A)

THE GERMAN CANCER RESEARCH CENTRE
in Heidelberg (Federal Republic of Germany)

invites applications for the post of

HEAD OF THE DIVISION
(Abteilungsleiter)

MOLECULAR BIOLOGY OF THE CELL

The applicant should have experience in research on structures of the nuclear genome and/or the regulation of gene activity at the molecular level.

Preference will be given to candidates with experience in methods of isolation and cloning of genes and gene fragments.

The applicant should be prepared to cooperate closely with other divisions in the German Cancer Research Centre; modern laboratory facilities and access to central services are provided.

Applications, together with a list of publications, should be sent by April 30, 1979 to:

Deutsches Krebsforschungszentrum,
Stiftungsvorstand, Im Neuenheimer Feld 280,
6900 Heidelberg 1, Federal Republic of Germany.
9742(A)

Veterinary Research

The Ministry of Agriculture, Fisheries and Food needs Research Officers in its veterinary laboratories at Weybridge and Shinfield. The main work of these laboratories is research into animal diseases and the means of dealing with them: examinations in connection with scheduled diseases and official control schemes are also carried out and certain biological products are prepared. Newly appointed Research Officers normally spend part of their first two years in training. During their careers they will broaden their experience by working on a variety of research subjects.

Central Veterinary Laboratory, Weybridge

In the Departments of Biological Products and Standards, Biochemistry, Diseases of Breeding, Bacteriology, and Virology.

Cattle Breeding Centre, Shinfield

For research into cattle and pig fertility and problems of breeding.

Candidates should normally be members of the Royal College of Veterinary Surgeons or expect to qualify in 1979, but candidates with an honours degree in an appropriate science subject may be considered, providing they have had at least 2 years' postgraduate practical experience in work relevant to veterinary science.

Appointment will be as Research Officer (Veterinary Service) with salary starting between £5,720 and £7,020, according to qualifications and experience, and rising to £8,240; Shinfield figures £275 less. There are prospects of promotion to posts carrying salaries of £9,500 and above. Non-contributory pension scheme.

For further details and an application form (to be returned by March 23, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote S/5077/4. 9714(A)

UNIVERSITY OF DAR ES SALAAM TANZANIA

Applications are invited for the following posts in the

DEPARTMENT OF ZOOLOGY

LECTURER/SENIOR LECTURER IN MARINE BIOLOGY (Fish Taxonomy/Aquaculture)

Applicants should hold a Ph D. with several years research and teaching experience in Fish Taxonomy and/or aquaculture Appointee will be required to organise undergraduate courses in Fish Taxonomy and/or aquaculture and to teach and supervise research in his/her subject area at postgraduate level, to organise and carry out research in fish taxonomy and biology. He/she will have to work in collaboration with other members of the Department in an integrated research programme

LECTURER/SENIOR LECTURER IN MARINE BIOLOGY (Marine Ecologist)

Applicants should have a Ph D. with several years research and teaching experience in Benthic Ecology/Coral reef ecology of tropical waters. The appointee will be required to organise undergraduate courses in Marine Ecology and to teach in his/her area of specialisation at postgraduate level; to plan and carry out research in Benthic Ecology/Coral reef ecology He/she will have to work with other members of the Department in an integrated research programme.

LECTURER/SENIOR LECTURER IN MARINE BIOLOGY (Fisheries Biology/Population Dynamics)

Applicants should hold a Ph.D. with research and teaching experience in Fisheries biology especially in the area of fish population dynamics The appointee will be required to organise undergraduate courses in Fisheries biology and to teach and supervise research in his/her subject area at postgraduate level, to organise and carry out research in Fisheries biology relevant to Tanzania He/she will be expected to work in collaboration with other members of the Department in an integrated research programme.

Salary Scales (Salaries are exclusive of 50% expatriation allowance). Senior Lecturer £T1,980 to £T2,480 p a, Lecturer £T1,620 to £T1,980 p a (£T1=£1 22 sterling.) The British Government may supplement salaries in range £4,356 to £4,782 p a (sterling) for married appointees and £2,754 to £3,018 p a (sterling) for single appointees (reviewed annually and normally free of tax) and provide children's education allowances and

Detailed applications (2 copies) with curriculum vitae and naming holiday visit passages. Family passages, F S S.U., biennial overseas leave. 3 referees to be sent direct to Chief Academic Officer, University of Dar es Salaam, P O. Box 35091, Dar es Salaam, Tanzania, by April 8, 1979.

Applicants resident in the U K should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address 9798(A)

UNIVERSITY OF AUCKLAND
New Zealand

Applications are invited for the following full-time teaching position Conditions of Appointment and Method of Application are available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. Applications in accordance with 'Method of Application' will be accepted at any time up to March 31, 1979.

Commencing salary in accordance with qualifications and experience will be established within the range for Lecturers or Senior Lecturers, at present NZ$11,529 to NZ$16,780 per annum.

ANALYST IN GEOCHEMISTRY
Lecturer status:

The position would suit a graduate with a research degree in Geochemistry or in suitable aspects of Mineralogy and Petrology, and with training and experience in both X-ray flourescence and wet chemical techniques. Applicants should note that analytical equipment in the Geology Department includes XRF, AA, DTA, TGA, IR, XRD, X-ray cameras, microprobe, PDP8 computer, and HP10 calculator and XY plotter, with access to campus facilities for SEM and B6300 and Prime computer systems.

Duties are concerned with the departmental research programme and instruction of senior students in analytical techniques and related topics. The major component of this work will be bulk rock and trace element analyses of selected samples, and some water analyses. The Analyst will be able to function as a co-worker in some of these research topics. In addition, it is expected that the Analyst will develop a personal research programme in Geochemistry as a second priority. 9781(A)

UNIVERSITY OF EDINBURGH
THE EDINBURGH SCHOOL OF AGRICULTURE
UNIVERSITY DEMONSTRATOR IN ANIMAL PRODUCTION

Applications are invited from suitably qualified graduates for the post of UNIVERSITY DEMONSTRATOR IN ANIMAL PRODUCTION This is a new post and is tenable for a period of up to three years.

The successful candidate will be involved in demonstrations and practical classes with farm animals for postgraduate, graduate and H.N.D. students. There will be involvement with lecturing and an opportunity to develop research interests.

Salary scale· £3,384 to £4,882 per annum.

Further details are available from the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL to whom applications should be sent not later than March 15, 1979. Please quote Reference 1022. 9769(A)

UNIVERSITY OF MIAMI
DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

Applications are invited for a

POSTDOCTORAL RESEARCH ASSOCIATE

to work with Dr D W Ribbons o
the biochemical and genetic organiz
tion of catabolic apthways in bacteri
Previous experience in pathways of ar
matic catabolism and/or mapping
plasmid genes by *in vivo* and *in vit*
techniques will be an advantage.

Applications, which should inclu
a curriculum vitae and the nam
and addresses of three referees shou
be sent by April 20, 1979 to Dr D
Ribbons, Biochemistry, UMED, P
Box 016129, Miami, Fl 33101 US
from whom further particulars may
obtained. An equal opportunity/affir
ative action employer 9739(A)

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MEDICAL RESEARCH COUNCIL
MAMMALIAN DEVELOPMENT UNIT
UNIVERSITY COLLEGE LONDON

A vacancy exists for a researc
scientist with postdoctoral experienc
in mammalian development a
genetics, to work on problems of
vitro culture and chromosomal anal
sis of mouse and human embryon
material, starting October, 1979 Th
position is a 3-5 year limited-ter
appointment on the M.R.C. no
clinical scientific staff, with salary o
the Grade II scale (£3,883 to £6,08
according to age and experienc
Applicants should send a curriculu
vitae and names of two referees t
Dr A McLaren, Director, M R.
Mammalian Development Un
Wolfson House, 4 Stephenson Wa
London NW1 2HE, by March 1
1979. 9770(A)

UNIVERSITY COLLEGE LONDO
Research assistant requird to work
M.R.C.-supported project studyi
binding of radio-labelled ligands
purified synaptic membranes. Ph.D.
equivalent experience in releva
methodology desirable. Applications
Dr C. C. Jordan, Department
Pharmacology, University Colle
London, Gower Street WC1E 6BT
soon as possible. 9741(A)

UNIVERSITY OF BRADFORD
LECTURER/ SENIOR LECTURER IN PHYSICS

Applications invited from suitably qualified physicists for a permanent post which may be filled at either Lecturer or Senior Lecturer level The research interests of the School of Physics lie in Physical Electronics and Nuclear Structure Physics, and applicants should be well qualified to pursue research in one of these fields Salaries within the ranges Lecturer — £3,883 to £7,754 p a., Senior Lecturer — £7,506 to £9,263 p a Further details/application forms from the Registrar, Post Ref PY/L/27/X, University of Bradford, BD7 1DP They should be returned by March 19, 1979

9711(A)

UNIVERSITY OF BRISTOL
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a

OSTDOCTORAL ASSISTANT POST (RANGE 1A)

join a project funded by the edical Research Council to develop tomatic protein sequencing techiques in relation to three projects in is Department The projects involve nino acid sequence studies on ovoansferrin (Dr J. Williams), glycolytic zymes (Dr H Muirhead) and the ythrocyte membrane anion transport otein (Dr M J. A Tanner), and it hoped that the applicant will also come actively involved in one of em

The post is tenable for 2 years and available immediately Some previous perience in protein chemistry and nino acid sequence methodology ould be desirable.

Applicants should send a copy of eir curriculum vitae and the names two academic referees to Dr J A Tanner, Department of Biochemistry, University of Bristol, istol BS8 1TD, as soon as possible

9793(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL
DEPARTMENT OF MEDICINE
POSTDOCTORAL IMMUNOLOGIST

quired to work in the Immunological edicine Unit, on the role of comement in the induction of immunogical responses in man and experiental animals The project is funded y the Medical Research Council and for two years in the first instance

Salary on the scale £3,910 to £4,658 lus £450 London Allowance, initial acing dependent on experience and ualifications

Further details may be obtained om Dr M. B. Pepys, Department of edicine, Royal Postgraduate Medical chool, 150 Du Cane Road, London 12 0HS

Application forms to be returned to e Personnel Office, 150 Du Cane oad, London W12 0HS.

9722(A)

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e and postdoctoral positions are availle for studies on the protein synthesing system with special emphasis on ructure and function of mRNP rticles, ribosomes—topography, biomesis and function—under various nditions of growth and development Teaching opportunities available, oply with resumé to Professor ruce H. Sells, Laboratories of olecular Biology, Faculty of Medine, Memorial University of Newundland.

9751(A)

Wellcome

Senior Technician

BIOCHEMICAL RESEARCH FROM £3,400

to join the Biochemical Research Department of The Wellcome Research Laboratories, located at our main manufacturing site in Dartford, Kent.

The successful candidate will assist a scientist in endocrinological research, in particular on the mechanisms of polypeptide hormone secretion

Candidates should be qualified to H.N.C. level in biology/chemistry or equivalent and have approximately two years' relevant experience. There will be an opportunity for the successful candidate to progress by day-release to a B.Sc. degree

The Wellcome Foundation Limited is a major British-owned international pharmaceutical organisation producing a wide range of chemical and pharmaceutical products

Excellent benefits include 4 weeks' holiday, pension, life assurance and sick pay schemes, subsidised restaurant, first class sports and social facilities and generous assistance with relocation expenses, where appropriate.

For further details and an application form, please write to or ring
Ian Griffiths, Personnel Division,
The Wellcome Foundation Limited,
Dartford, Kent.
Tel: Dartford (32) 23488, ext. 2061.

9731(A)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post of

LECTURER IN BIOCHEMISTRY

tenable from October 1979.

The successful candidate will be expected to be interested in mammalian and medical biochemistry and to participate in teaching and research in these areas A medical degree would be an advantage although is not essential The Department provides courses for science, medical and dental undergraduates Excellent facilities are available for most types of biochemical research

Salary within the range £3,883 to £7,754 p a (plus USS), depending on age, qualifications and experience

Applications (2 copies) naming 3 referees should be sent by March 30, 1979 to Miss L R Heyes, Senate Registry, University of Birmingham, P O. Box 363, Birmingham B15 2TT, from whom further particulars may be obtained

9779(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF OCEANOGRAPHY

Applications are invited for the post of Lecturer in the Department of Oceanography. The post is tenable immediately

The person appointed will be expected to teach and carry out research in Marine Chemistry

The initial salary will be within the range £3,883 to £5,367 per annum on a scale rising to £7,754 per annum.

Applications, together with the names of three referees, should be received not later than March 22, 1979 by The Registrar, The University, P.O Box 147, Liverpool L69 3BX, from whom further particulars may be obtained Quote Ref RV/498/N

9777(A)

Director

National Center For Toxicological Research Food and Drug Administration

The U.S Food and Drug Administration is seeking candidates for the position of Director of the National Center for Toxicological Research (NCTR) The Center, an interagency facility, conducts research programs on the biological effects of potentially toxic chemical substances found in the human environment The Director of NCTR provides leadership and direction to all Center operations and provides scientific liaison between NCTR and other Government agencies, private organisations and the worldwide academic, scientific and medical communities The Center is located in Jefferson, Arkansas and has a staff of approximately 600 employees The Center is well-equipped with sophisticated and modern laboratories to support advanced research in virtually all areas of toxicology

FDA is looking for candidates of recognised scientific distinction and administrative ability Candidates should possess an advanced degree in a relevant field of science (such as a Ph D in biochemistry, pharmacology, toxicology, or statistics, an M D., or a D V M.) The position also requires appropriate research experience and an association with multidisciplinary issues This is a Civil Service position that pays up to $47,500 per year in basic salary. The Director will also be eligible to receive performance bonuses and special stipends that may result in a higher total pay This position has been designated for inclusion in the Federal Government's Senior Executive Service (SES), and the selectee will be required to enter the SES. Information on the SES will be available for interested applicants

Send curriculum vitae and/or Standard Form 171 (Personal Qualifications Statement) and bibliography to

HEW/Food and Drug Administration,
NCTR Search Committee,
c/o Ms. Patricia Sykes,
Rm. 9-89, 5600 Fishers Lane,
Rockville, Maryland 20857, U.S.A.

FDA/An Equal Opportunity Employer.

9784(A)

Microbial Physiologist

Overseas Assignment

The Kuwait Institute for Scientific Research, a rapidly expanding institute for applied science and technology, is seeking a Microbial Physiologist who is interested in the opportunity for a career challenge

In this position, you will be responsible for the yield coefficient and productivity studies of SCP production from LPG and methanol in bench-scale fermenters.

We require a PhD in Microbiology or Biochemistry with at least 3 years' experience in continuous culture techniques, microbial growth efficiency or hydrocarbon microbiology.

Kuwait Institute offers attractive salaries commensurate with qualifications and experience, furnished housing, liberal fringe benefits including round trip air tickets and free medical benefits.

Please airmail complete resume by April 1, 1979 to: **Mr. Habib Al-Sahhaf, Personnel Manager, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat, Kuwait, State of Kuwait.** 9703(A)

Kuwait Institute for Scientific Research

CSIRO AUSTRALIA

Postdoctoral Research Fellow

Division of Land Resources Management, Perth, WA

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees —2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia

Field: Coastal Dynamics

General The Division has 60 professional staff doing problem-oriented research on the environmental, societal, economic and technological implications of land use One program includes studies of the dynamics of both ocean and land in the coastal zone

Duties. The appointee will work in a multidisciplinary team and will be expected to develop a research program on the prediction of sea level oscillations on the coastline and the energetics of the coastline Other areas of interest are sediment motion and shore erosion, and transport of sand by wind

Qualifications: A PhD or equivalent research experience in applied mathematics, engineering, physical oceanography or physics The ability to relate scientific analysis to problems of resource management is essential.

Salary: Research Scientist or Senior Research Scientist $A15,422 to $A22,405 p a An outstanding applicant may be offered appointment at a higher level

Tenure: A fixed term of three years

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 284/91 should reach

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 21st March 1979

Applications in U S A and Canada should be sent to. The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W , Washington, D.C , 20036, U S.A 9775(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF GENETICS

Applications are invited for the temporary post of

POSTDOCTORAL RESEARCH FELLOW

in the above Department The successful candidate will participate in current research programmes involving the cloning of fungal and bacterial DNA and will be using techniques that include nucleic acid hybridisation, heteroduplex mapping and separation and mapping of restriction fragments Familiarity with some or all of these would be an advantage, but persons with related biochemical experience are encouraged to apply. Candidates should possess a Ph D degree The appointment will be made for a fixed term of three years with effect from October 1, 1979

Salary at an appropriate point on the IA Scale for Research and Analogous Staff (£3,883 to £6,555) (under review), according to age, qualifications and experience

Informal enquiries about the post may be addressed in the first instance to Dr S Baumberg (ext 597) or Dr J C Wootton (ext. 7155) at the University (tel (0532) 31751).

Application forms and further particulars of the post may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 50/4/D

Closing date for applications March 31, 1979 9787(A)

UNIVERSITY OF ESSEX

CHAIR IN BIOLOGY

Applications are invited for a chair in Biology for appointment from October 1, 1979. The Chair will be filled in the fields of Developmental/Cell Biology, or microbial/molecular genetics

Applications (12 copies), including a curriculum vitae and the names and addresses of three referees, should reach the Registrar (C/1), University of Essex, Wivenhoe Park, Colchester CC4 3SQ, from whom further particulars may be obtained, by March 15, 1979 9716(A)

BIOCHEMICAL SOCIETY

Applications are invited for a senior post in the Society's Editorial Office The post involves responsibility for editorial processing of material for the **Biochemical Journal** and other Biochemical Society publications, and the successful applicant will be required to act as a deputy for the Editorial Manager As well as a degree in biochemistry or related subject it is essential that candidates (probably of age 35-45) should have had sound postgraduate experience of scientific editorial work and academic publishing and be capable of supervising the work of others, A salary commensurate with the seniority of this post will be offered Please apply in writing, giving the names of two referees, to the Eexcutive Secretary, Biochemical Society, 7 Warwick Court, London WC1R 5DP

9622(A)

FRIEDRICH MIESCHER-INSTITUT

seeks

EXPERIENCED PLANT CELL BIOLOGIST

to form and lead a group doing basic research on morphogenesis from single cells of corn and other cereals.

Applications, before April 1, 1979, to Dr R. R. Bürk, Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland 9735(A)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitab qualified persons for appointment the following positions

THE JOHN CURTIN SCHOOL O MEDICAL RESEARCH.

PROFESSORIAL FELLOW IN NEUROCHEMISTRY

DEPARTMENT OF PHARMACOLOGY

Applications are invited from neur chemists with recognised experien and expertise in the chemistry central synaptic transmission in t mammal for appointment as Pr fessorial Fellow in Neurochemistr The successful applicant will expected to lead a small neur chemical group within the Departme of Pharmacology and to take advanta of collaboration with other membe of the Department engaged on *in vi* investigations of central synapt mechanisms, particularly those invol ing amino acid and peptide tra mitters Further details may obtained from Professor D R Cur Head of Department

Closing date **April 13, 1979.**

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH FELLOWS

DEPARTMENT OF BEHAVIOURAL BIOLOGY

Two positions are available. App cants should have a higher degree a postdoctoral experience in expe mental psychology or animal behavio Work in the Department is mult disciplinary and has concentrated the development of the visual syste particularly in bird brains, and on t mechanisms of memory storage. F further details please contact Profes Richard Mark, Head of Departme

Closing date: **April 15, 1979.**

POSTDOCTORAL FELLOW

MOLECULAR BIOLOGY UNIT

The Unit (Head, Dr H Naora) exploring the molecular mechanis of post-transcriptional control animal cells This includes t problems of the HnRNA-mRNA p cursor-product relationship, the nucle processing of mPNA-precurs molecul using nucleic acid sequencing a DNA cloning Applicants should ha experience in this particular field research

Closing date **April 15, 1979.**

Salaries Professorial Fellow, $A30,7 p a , Research Fellow, in accorda with qualifications and experien within the range $A15,786 to $A20,6 p a , Postdoctoral Fellow, a fixed po within the range $A15,786 to $A20, p a Current exchange rates $A £0 56 sterling

Term of Appointment: Professor Fellow to retiring age (65 years), search Fellow for three years in first instance with the possibility extension to five years, Postdocto Fellow for two years

Other Conditions: Reasonable appoi ment expenses are paid Superannuat benefits are available for applica who are eligible to contribute Ass ance with housing is provided for appointee from outside Canberra

The University reserves the right to make an appointment or to ma an appointment by invitation at time

Prospective applicants should obt the further particulars from Association of Commonwealth U versities (Appts), 36 Gordon Squ London WC1H 0PF 9799(A

RESEARCH GROUP requires labo tory scientific officer to assist genetic and cell biology work invo ing tissue culture. Candidates w relevant experience preferred. Ap cations should be sent to the Admi strative Assistant, Paediatric Resea Unit, The Prince Philip Resea Laboratories, Guy's Hospital Med School, London Bridge SE1 9RT 9709(A

THE MAX-PLANCK-INSTITUTE OF BIOCHEMISTRY
8033 Martinsried/Munich

offers a postdoctoral position for a biochemist, preferentially with cell biological experience in the field of membrane proteins. Payment as research assistant (ca DM3,000, monthly).

Applications to Prof. E. Neumann, Max-Planck-Institut fur Biochemie, D-8033 Martinsried bei Munchen, Germany. 9748(A)

Please mention
Nature
when replying to
these advertisements

FELLOWSHIPS

Research Fellowships

St. Catherine's College and St. Hilda's College, Oxford University

The Rutherford Laboratory of the Science Research Council, St. Catherine's College and St. Hilda's College invite applications for two joint Research Fellowships, one at St. Catherine's College and one at St. Hilda's College. The aim of these "Atlas Fellowships" is to encourage theoretical studies – particularly with reference to those involving computers – in an area of research supported by the Rutherford Laboratory. These include the following broad fields: study of condensed matter by scattering of neutron beams; study of dense plasmas by the use of intense laser beams; use of electron beam lithography in fabrication of semi-conductor devices. The successful candidates will be expected to undertake independent research work and to have ideas on the programme they wish to follow.

The Fellows will have the rights and privileges of a Research Fellow to the relevant College. The possibility may exist for the Fellow to undertake some teaching. They will be expected to spend about half their time at the Rutherford Laboratory and will be provided access to exceptionally powerful computing facilities. The Rutherford Laboratory's involvement with numerous University research groups gives an opportunity for contacts with other research workers engaged on a wide range of exciting research programmes.

The Fellowship will be tenable for a period of up to three years and may be extended up to a period not exceeding five years. The stipend will depend upon the age and experience of the candidates selected within the broad range £4,500 to £7,500. (The stipends are currently under review). The Fellowship will carry the superannuation provisions under the SRC Principal Superannuation Scheme which is a non-contributory scheme. Candidates will normally be expected to take up the posts in October 1979.

For details of Research Fellows' rights at the two Colleges and application forms, write to the Personnel Group of the Rutherford Laboratory, Chilton, Didcot, Oxon OX11 0QX quoting reference No. VN 730.

The closing date for applications is 16th March, 1979.

RUTHERFORD
Science Research Council
Rutherford Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX. Tel Abingdon 21900

9780(E)

DEPARTMENT OF EDUCATION, DUBLIN
POSTDOCTORAL RESEARCH FELLOWSHIPS
1979–80

Applications are invited for Research Fellowships in Science (excluding Medical and Social Science) and Engineering tenable in any one of the following Institutions in Ireland University College, Dublin, University College, Cork, University College, Galway, Trinity College, Dublin, An Foras Talúntais (The Agricultural Institute), Dublin 4, The Institute for Industrial Research and Standards, Dublin 9 Fellowships will be tenable for a period of two years with the possibility of an extension for a third year

Applicants should hold the degree of Ph D or have equivalent research experience Applications will be considered from engineers or technologists with suitable industrial experience Candidates should not be more than 30 years of age but, preferably, they should be under 28 years of age

Appointments will be effective from a date not earlier than October 1, 1979 The initial salary, which will depend upon qualifications and experience, will normally be within the range £3,775 to £4,277 per anum, but awards of up to £4,986 may be made in the case of engineers and technologists whose industrial experience or other special circumstances warrant it

Full particulars of the scheme including application forms may be obtained from the Secretary, Department of Education (Headquarters Section 3), Marlboro' Street, Dublin, 1, to whom applications for Fellowships should be forwarded The latest date for the receipt of applications is March 20, 1979 9786(E)

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)
S.R.C. C.A.S E.
STUDENTSHIP

Applications are invited from graduates or those who will graduate in 1979 for an S.R.C C.A.S.E. Studentship held jointly between the Biology Department, Queen Elizabeth College, and the Centre for Overseas Pest Research, London. The project is concerned with host plant resistance to aphids and is specifically linked with Sorghum crops. Applicants should hold or expect to obtain at least an upper second class honours degree in a biological subject.

Further details can be obtained from Dr M Llewellyn, Biology Department, Queen Elizabeth College, Campden Hill Road, London W8 7AH.

9763(F)

UNIVERSITY OF EDINBURGH
FACULTY OF MEDICINE
POSTGRADUATE FELLOWSHIPS

Applications are invited from medical or dental graduates wishing to undertake work towards a higher degree, and from those already holding Ph.D. degrees, for Faculty of Medicine Fellowships tenable from October 1, 1979. The awards include a stipend of £4,250, the payment of University fees and assistance towards conference expenses.

Further details and application forms, which must be returned by March 16, 1979, may be obtained from the office of the Dean of the Faculty of Medicine, University of Edinburgh, The Medical School, Teviot Place, Edinburgh EH8 9AG.

9774(E)

FEBS FELLOWSHIPS

FEBS Fellowships are to support short-term (not longer than two month) visits by members of any FEBS Constituent Society to laboratories in another country in the FEBS area for the purpose of carrying out experiments with special techniques or other forms of scientific collaboration or advanced training, and especially to support developments arising at short notice.

Fellowships will *not* be granted to attend courses, symposia, meetings or congresses.

Fellowships will cover both travel and subsistence.

Information and details of making an application may be obtained from:

Professor G. Dirheimer,
FEBS Fellowships Officers,
Institut de Biologie Moléculaire et Cellulaire du CNRS,
15 rue Descartes,
67084 Strasbourg Cedex,
France.

9754(E)

UNIVERSITY COLLEGE DUBLIN
DEPARTMENT OF HORTICULTURE
POSTDOCTORAL FELLOWSHIP

Applications are invited for a postdoctoral fellowship, sponsored by The National Board for Science and Technology, to study protein degradation and reutilisation in fruit trees and germinating seeds.

Applicants should have relevant research experience in the fields of horticulture, plant physiology or biochemistry.

The appointment will be for one year, initially, beginning as soon as possible. The starting salary will be £3,837 per annum on an incremental scale.

Applications giving details of qualifications and experience and the names and addresses of two referees should be sent to.

Dr M. J. Hennerty,
Department of Horticulture,
University College,
Glasnevin,
Dublin 9.

9782(E)

UNIVERSITY OF ABERDEEN
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a Postdoctoral Research Fellow to work with Dr F. P Glasser on energy reduction in cement clinkering. A background in solid state or physical chemistry, ceramics, materials science or petrology-mineralogy would be advantageous

The post is supported by the Science Research Council for two years Starting date by arrangement within the period May–August 1979 Salary on Research and Analogous Staff IA Scale, £3,883 to £4,882, per annum, with appropriate placing

Further particulars from The Secretary, The University Aberdeen with whom applications (2 copies) should be lodged by March 16, 1979

9746(E)

UNIVERSITY OF BRADFORD
RESEARCH FELLOW IN VISUAL SCIENCE

Applications are invited for a 2 year Post-Doctoral Fellowship for research on the Characteristics of Normal and Defective Colour Vision. The successful candidate will be attached to the new Chair in Visual Science and will work under the direction of Professor J D. Moreland. It is hoped to make the appointment as soon as possible. Salary within range £3,883 to £6,555 p.a (under review) Superannuable. Further particulars and application forms (to be returned within 14 days of this advertisement) from the Registrar, Post Ref VS/RF/X, University of Bradford, BD7 1DP, West Yorkshire

9712(E)

UNIVERSITY OF CAMBRIDGE
POSTDOCTORAL RESEARCH FELLOWSHIP
IN THE DEPARTMENT OF METALLURGY AND MATERIALS SCIENCE

A postdoctoral research fellow is required to investigate ordering and precipitation reactions in tungsten-based alloys, using primarily scanning/transmission electron microscopy and high-voltage electron microscopy The post will initially be for a period of two years commencing on October 1, 1979. The starting salary will be according to age and experience but is expected to be within the lower half of the "Agreed Scales" for a University Lecturer, at present £3,975 to £8,250 (under review), with full USS supplementation Applications including a curriculum vitae should reach The Secretary, University of Cambridge, Department of Metallurgy and Materials Science, Pembroke Street, Cambridge, CB2 3QZ, not later than March 16, 1979

9783(E)

COURSES

UNIVERSITY OF LEICESTER
M.Sc. IN EXPERIMENTAL SPACE PHYSICS

Applications are now being considered for the Space Physics course for entry in October. Candidates should have a degree with 1st and 2nd class honours in Physics, Mathematics or Engineering The course runs for 12 months and includes lectures, dissertation and project work covering both scientific (astronomy, earth sciences, applications) and technical (propulsion, orbits, communications, etc) aspects of present-day space research. A number of SRC studentships are available for successful applicants

Write for further details, including 2 academic referees, to Professor K A Pounds, Dept of Physics, University of Leicester, Leicester LE1 7RH.

9778(D)

M.Sc. in Medical Imaging Royal Free Hospital School of Medicine and Institute of Cancer Research (University of London). This is a new part-time course, lasting two calender years, commencing in October 1979, in which the formation, perception and analysis of images of biological structures will be considered, with particular reference to *in vivo* studies in medicine The course will be of particular value to persons working in hospitals or in employment with medical interests.

Applicants should normally have a First or Second Class Honours degree in physics, mathematics or a physical science.

Details of fees and application forms from: Professor H. A. B. Simons, Academic Department of Medical Physics, Royal Free Hospital, Pond Street, London NW3 2QG. 9767(D)

Ph.D. IN TOXICOLOGY

Applications are invited for a postgraduate course in Toxicology fo
which M.R.C postgraduate grants are available. Entrance requirement
are as for a normal Ph.D. The first year is a full-time course leading t
an M.Sc To give opportunities for further research training in toxicolog
the grants awarded for the M Sc will be extended for those student
showing satisfactory progress in the first year. These grants would be fo
three or four years in total as appropriate.

The Medical Research Council, the Royal Society and the Roy
College of Pathology have identified toxicology as a priority area fo
research training and therefore support the encouragement of first cla
students from a variety of disciplines to become involved in the scienc
of toxicology. In modern society contact with potentially toxic chemical
is increasing. Problems may arise from the use of drugs for medica
purposes, from occupational exposure to industrial products or inte
mediates and from environmental exposure to natural or syntheti
substances.

The postgraduate course in Toxicology, which was the first of its kin
in Europe, is organised by the Department of Biochemistry, University o
Surrey, with the M.R.C. Toxicology Unit, Carshalton, the Britis
Industrial Biological Research Association, Carshalton and Shell Tox
cology (Tunstall) Laboratory, Sittingbourne. During the course instructio
is given in these collaborative laboratories as well as at other majo
toxicological institutions.

The course is designed to provide graduates in science, and medical an
veterinary graduates, with an understanding and appreciation of the man
disciplines involved in toxicology Particular emphasis is placed on aspec
related to the biochemical mechanisms of chemically-induced toxicity, an
the assessment of toxicological hazard in the manufacture, use an
disposal of new chemical compounds, pesticides and medicines The cours
provides a broad biological education and is a suitable introduction fo
those intending to undertake research on the interaction of chemicals wit
biological systems.

The research period for the Ph.D. may be undertaken in one of th
above organisations or in other laboratories approved by the University
being suitable for toxicological research.

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initially in different disciplines, who have acquired additional knowled
of toxicology. These may be in industry, government regulatory bodies,
research institutes with good career prospects in both laboratory work an
administration.

Further information and application forms to be returned by March 2
1979, may be obtained from·

Dr R. S. Jones,
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9749(D)

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Formalism and will go through the theory of Spin Relaxation. The rece
developments on the instrument side will be shown and the princip
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topics in the field of metalloenzymes, metalloproteins and of interactio
of nucleic acids with metal ions will be covered. On request of t
participants, microcourses will be held with the purpose to fill the g
in the theoretical foundations of the magnetic resonance experime

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F Wehrli (Varian Zug), K. Wuthrich (Zurich) I. Bertini (Florenc
R S Drago (Urbana), Directors

A limited number of grants are available to participants from N.A T
countries They will cover about 50% of the living expenses and 'ab
25% of the travel expenses Living expenses are 276,000 Italian Li

Applications addressed to :

Prof. I. Bertini
Istituto di Chimica Generale della Facolta di Farmacia
Via G. Capponi 7
50121 FLORENCE, ITALY

may be made in the form of a letter giving a short curriculum vitae
present scientific interest.

9803(D

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9789(D)

FEBS ADVANCED COURSES

No. 65. "The Control of Glycogen Metabolism"
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5-7 September 1979

The Course, organised by H. De Wulf, H. G. Hers, L. Hue and G. van de Werve, will cover the properties of enzymes involved in glycogen metabolism as well as aspects specific to muscle, liver, adipocytes and leucocytes. It will include lectures by invited speakers and presentation of posters by registered participants. A limited number of these posters will be subjected to general discussion. Registration fee: about $50. For further information, contact: Professor H. G. Hers, Université Catholique de Louvain and International Institute of Cellular and Molecular Pathology, UCL 75.39-75 avenue Hippocrate, B-1200 Brussels, Belgium. The deadline for applications and abstracts is **May 15, 1979.**

No. 57. "Recombinant DNA"
Zagreb, Yugoslavia,
15-27 October 1979

A Laboratory Course for 25 participants. The experimental program will include: In vitro recombination of DNA segments with plasmid and viral vectors; Cloning in *Escherichia coli* and *Bacillus subtilis;* Selection and screening of cloned recombinant DNA; Characterization of cloned recombinant DNA. Registration fee: $100. Applications **(by April 30, 1979)** and info: Dr Vjera Zgaga, Dept. of Experimental Biology and Medicine, "Ruder Boskovic" Institute, P.O.B. 1016, 41001 Zagreb, Yugoslavia.

No. 64. "Modern Methods of DNA Sequencing"
Gliwice, Poland,
5-10 November 1979

This Course for 15 students aims to disseminate within European research laboratories the two basic methods of DNA sequencing: the Maxam-Gilbert technique and Sanger's inhibitor chain-termination technique. Advanced studies will cover sequence organization of DNAs from various sources. Further information and application forms (to be returned **by May 31, 1979)** are available from the organizers: Dr M. Chorazy/Dr S. Szala, Dept. of Tumor Biology, Institute of Oncology, 44-100 Gliwice, Poland.

9753(D)

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Further information and application forms from: The Secretary, Department of History and Philosophy of Science, Chelsea College, Manresa Rd, London SW3 6LX (Tel: 01-352 6421).

9805(D)

STUDENTSHIPS

UMIST
S.R.C. C.A.S.E. STUDENTSHIP

Applications are invited for a C.A.S.E. Studentship financed by S.R.C. in collaboration with Shell Biosciences Laboratory, Sittingbourne, tenable for three years, for a study of the effects of high pressure/temperature enviornments on the physiology, biochemistry and corrosive properties of sulphate-reducing bacteria using already developed culture systems.

The study would be of particular interest to those with a background in microbiology, applied biology or biochemical engineering, though candidates with other qualifications will be considered.

A number of C.A.S.E. awards in other areas of corrosion science are also expected to be available and general applications will be considered.

Applicants should send curriculum vitae and names and addresses of two referees to Professor G. C. Wood, Corrosion and Protection Centre, UMIST. Manchester M60 1QD.

9788(F)

UNIVERSITY OF EAST ANGLIA
SCHOOL OF BIOLOGICAL SCIENCES
POSTGRADUATE STUDENTSHIPS

Applications are invited for the following Science Research Council-funded studentships:

1. 'A study of mutational processes in myxobacteria': supervisor Dr C. H. Clarke
   or
   'The role of asparagine in the nitrogen metabolism of plants': supervisor Dr A. P. Sims.
2. 'Molecular enzymology of terminal respiratory proteins in bacteria and/or mammals': supervisor Dr C. Greenwood.
3. 'Transport processes in artificial bilayer lipid membranes': supervisor Dr E. J. A. Lea
   or
   'Transport processes in plant leaves': supervisor Dr J. F. Thain.
4. 'Resource partitioning among the fish populations in a Norfolk Broad': supervisor Dr C. R. Townsend.
5. 'Microtube function in cell formation, division and movement in *Drosophila* embryos'; supervisor Dr R. M. Warn.

Applicants should posses, or expect to obtain a First or Upper Second Class degree and should write to Professor A. F. G. Dixon, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, enclosing a curriculum vitae and the names of two referees.

9730(F)

S.R.C. C.A.S.E. RESEARCH STUDENTSHIP
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Applications are invited from persons holding, or expecting to obtain, a good honours degree in physics for a C.A.S.E. studentship in theoretical solid state physics concerning electronic properties of defects in semiconductors, in collaboration with the Royal Signals and Radar Establishment, Malvern.

Further details are available from Dr M. Jaros, School of Physics, The University, Newcastle upon Tyne, NE1 7RU, to whom applications should be sent along with curriculum vitae and the names of two referees.

9729(F)

ASSISTANTSHIPS

UNIVERSITY OF OXFORD
DYSON PERRINS LABORATORY
POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a postdoctoral research assistantship supported by the S.R.C. to further develop and exploit a new method for measuring kinetic isotope effects (including those for 'heavy' atoms) with high accuracy. Preference will be given to candidates with experience in kinetics or in synthesis with stable isotopes.

The appointment is for two years, commencing October 1, 1979, or at an agreed date. The salary will be on the Research Support Grade Scale 1A, initially not higher than £4,130 p.a. (under review), plus U.S.S. benefits.

Applications (one copy), with curriculum vitae and the names and addresses of at least two referees (overseas applicants should ask referees to write directly to the address below) should be sent not later than March 31, 1979, to Dr M. J. T. Robinson, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, from whom further information may be obtained.

9801(P)

THE EAST OF SCOTLAND COLLEGE OF AGRICULTURE

Applications are invited for a

RESEARCH ASSISTANTSHIP

tenable for a period of 18 months from March 1979, to be employed in research on certain storage diseases of potato tubers, principally dry rot.

Candidates should have a good honours degree in Biology, Botany or Agricultural Botany, preferably with some experience in plant pathology.

The work will be carried out in the Plant Pathology Department under the supervision of Dr A. E. W Boyd.

Salary: £3,384 per annum.

Further particulars and application form from The Secretary, The East of Scotland College of Agriculture, West Mains Road, Edinburgh EH9 3JG.

Closing date for applications: March 8, 1979.

9768(D)

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9597(P)

AWARDS

CHELSEA COLLEGE
University of London
PHARMACY DEPARTMENT
S.R.C. C.A.S.E. AWARD

Applications are invited from graduates of chemistry, engineering (chemical or mechanical), pharmacy or physics with a first or upper second class honours degree, or undergraduates who expect to graduate in 1979, for an S.R.C. C.A.S.E. award tenable in the Pharmacy Department of Chelsea College University of London. The project involves a study of the factors involved in the processing of materials for the manufacture of a novel dosage form or drugs and will be closely associated with the industrial sponsor. For further details and application forms contact the academic supervisor, Professor J. M. Newton, Pharmacy Department, Chelsea College, University of London, Manresa Road, London SW3 6LX.

9721(N)

LECTURES

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on

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A limited number of N.A.T.O. Grants for partial support of participants are available.

Applications must be submitted as soon as possible to:

P. W. Sanford (Conference Director)
Mullard Space Science Laboratory
University College London
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Dorking, Surrey, U.K.

The Advisory Committee consists of P. Laskarides (Athens—Local Organiser), A. Dupree (U.S.A.), J. Hutchings (Canada).

The detailed scientific programme will be defined in early April.

Travel arrangements are through American Express, Travel Division, Place Rogier, 24—1000 Brussels, Belgium.

9807(K)

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Dr R W Arndt, Mettler Instrumente AG, Greifensee, Switzerland.

Some of the papers included in the programme:

Training of clinical laboratory personnel in the use and maintenance of automatic systems, **Dr L B Roberts**, Gartnavel General Hospital, UK.

An experiment in education for automatic analysis: the 1979 Chemical Society Summer School, **Dr D Betteridge**, University College of Swansea, UK.

Recent developments in flow injection analysis, **Dr J Ruzicka**, The Technical University of Denmark, Lyngby, Denmark.

DACOS – a new approach to kinetic analysis, **M Snook**, Clinical Research Centre, Harrow, Middlesex, UK.

Recent developments in automatic chromatography, **Dr P B Stockwell**, The Laboratory of the Government Chemist, London, UK.

The cost benefits of automated analytical systems, **J G Jones**, Wessex Water Authority, UK.

Economic techniques for evaluating automation alternatives, **T M Craig**, E I Du Pont de Nemours & Co, Wilmington, Delaware, USA.

Automation of radioimmunoassay and related analytical techniques, **Professor J Landon**, St Bartholomew's Hospital, London, UK.

Industrial applications of automation with particular reference to the InfraAlyzer, **Dr H Swann**, University of Nottingham, School of Agriculture, UK.

Extraction in continuous flow systems with examples from pharmaceutical analysis, **Dr B Karlberg**, Astra Pharmaceuticals AB, Sodertalje, Sweden.

Improved accuracy in automated chemistry through the use of reference materials, **Dr R F Coleman**, National Physical Laboratory, Teddington, UK.

The symposium fee will be £130 inc. VAT and this fee will include: a) documentation and abstracts of all papers; b) luncheon, tea, coffee etc. To register for this symposium and obtain further details fill in the coupon below.

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9807(C)

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For further information please address inquires to:

Prof. Giampaolo Velo
Istituto di Farmacologia Policlinico
Borgo Roma 37100 Verona (Italy)
Telephone 045-500408

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International Meeting on Inflammation
24-27 September, 1979. Verona, Italy

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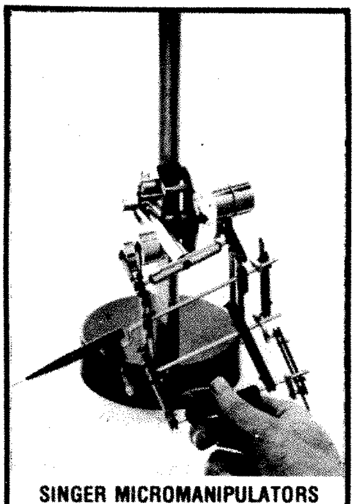

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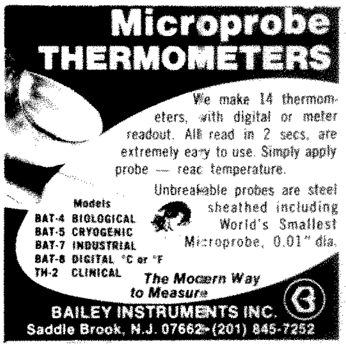

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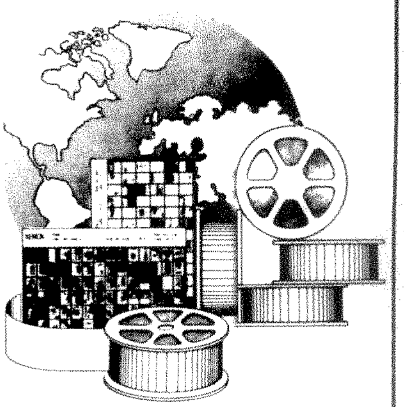

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